

FORM PTO-1390 U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE (MODIFIED)

ATTORNEY'S DOCKET NUMBER
X-11600

U.S. APPLICATION NO. (if known, see 37 C.F.R. 1.5)

10/018383

**TRANSMITTAL LETTER TO THE UNITED STATES
DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. 371**

INTERNATIONAL APPLICATION NO.
PCT/US00/06707

INTERNATIONAL FILING
DATE
05/09/2000 (05.09.00)

PRIORITY DATE CLAIMED
05/11/1999 (05.11.99)

TITLE OF INVENTION: AMYLOID PRECURSOR PROTEIN PROTEASE AND RELATED NUCLEIC ACID COMPOUNDS

APPLICANT(S) FOR DO/EO/US: Gerald Wayne Becker, et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
 - a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☐ has been transmitted by the International Bureau.
 - c. ☒ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
 - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☐ have been transmitted by the International Bureau.
 - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
 - d. ☒ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☒ A copy of the International Preliminary Examination Report (IPER), including any annexes, and, if not in English, an English language translation of the annexes to the IPER under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☒ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A **FIRST** preliminary amendment.
14. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
15. ☐ A substitute specification.
16. ☐ A change of power of attorney and/or address letter.
16. ☐ Other items or information:

U.S. APPLICATION NO. (if known, see 37 C.F.R. 1.5) 10/018383		INTERNATIONAL APPLICATION NO. PCT/US00/06707		ATTORNEY'S DOCKET NUMBER X-11600	
17.	<input checked="" type="checkbox"/>	The following fees are submitted:	CALCULATIONS PTO USE ONLY		
<p>BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)): Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO. \$1040.00</p> <p>International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO \$890.00</p> <p>International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO..... \$750.00</p> <p>International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) \$710.00</p> <p>International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) \$100.00</p>					
ENTER APPROPRIATE BASIC FEE AMOUNT =			\$ 890.00		
Surcharge of \$130.00 for furnishing the oath or declaration later than <u> 20 </u> <u> 30 </u> months from the earliest claimed priority date (37 CFR 1.492(e)).			\$		
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	22 -20=	2	X \$18.00	\$ 36.00	
Independent claims	9 -3=	6	X \$84.00	\$ 504.00	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ \$280.00	\$	
TOTAL OF ABOVE CALCULATIONS =				\$	
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28).				\$	
SUBTOTAL =				\$ 1430.00	
Processing fee of \$130.00 for furnishing English translation later than <u> 20 </u> <u> 30 </u> months from the earliest claimed priority date (37 CFR 1.492(f)).				\$	
TOTAL NATIONAL FEE =				\$	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31).				\$	
\$40.00 per property					
+					
TOTAL FEES ENCLOSED =				\$ 1430.00	
				Amount to be refunded	\$
				charged	\$
a.	<input type="checkbox"/>	A check in the amount of \$_____ to cover the above fees is enclosed.			
b.	<input checked="" type="checkbox"/>	Please charge my Deposit Account No. 05-0840 in the amount of \$ 1430.00 to cover the above fees. A duplicate copy of this sheet is enclosed.			
c.	<input checked="" type="checkbox"/>	The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 05-0840. A duplicate copy of this sheet is enclosed.			
<p>NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.</p>					
<p>SEND ALL CORRESPONDENCE TO: ELI LILLY AND COMPANY PATENT DIVISION/PKD LILLY CORPORATE CENTER</p>					
<p><u>Paula K. Davis</u> SIGNATURE</p> <p><u>Paula K. Davis</u> NAME</p>					
<p><u>10/30/2001</u> Date</p> <p><u>47.517</u> <u>(317) 433-3422</u> REGISTRATION NUMBER TELEPHONE NUMBER</p>					



PTO/PCT Rec'd 08 JUL 2002

#7

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Assistant Commissioner for Patents, Arlington, VA 22202, on the date appearing below.
ELI LILLY AND COMPANY

By Hinda M. DurbinDate July 2, 2002

PATENT APPLICATION
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	: Gerald Wayne Becker, et al.)	
Serial No.	: 10/018,383)	
Filed	: October 30, 2001)	Confirmation No.: 7903
For	: AMYLOID PRECURSOR PROTEIN)	
	: PROTEASE AND RELATED)	
	: NUCLEIC ACID COMPOUNDS)	
Docket No.	: X-11600)	

STATEMENT TO SUPPORT FILING AND SUBMISSION IN ACCORDANCE
WITH 37 C.F.R. 1.821(f) (SEQUENCE LISTING)

Assistant Commissioner for Patents
Arlington, VA 22202
Sir:

I hereby affirm that the content of the paper and computer readable copies of the Sequence Listing, submitted in accordance with 37 C.F.R. 1.821(c) and (e), respectively, are the same.

Respectfully submitted,

Paula K. Davis

Paula K. Davis
Agent for Applicants
Registration No. 47,517
Phone: 317-433-3422

Eli Lilly and Company
Patent Division
P.O. Box 6288
Indianapolis, Indiana 46206-6288

02 July 2002

107 10/018383

JC13 Rec'd PCT/PTO 30 OCT 2001

"Express Mail" mailing label number	<u>EL559725839 US</u>
Date of Deposit	<u>October 30, 2001</u>
I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. 1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.	
<u>Olga M Franz</u> Printed Name	<u>Olga M Franz</u> Signature

PATENT APPLICATION**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

The Accompanying Application

Applicants : Gerald Wayne Becker et al.

For : AMYLOID PRECURSOR PROTEIN PROTEASE AND
RELATED NUCLEIC ACID COMPOUNDS

Docket No. : X-11600

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents

Washington, D. C. 20231

Sir:

Please amend the accompanying application as follows.

In the Claims

Please cancel claims 1-22, and add the following new claims 23-44.

--23. An isolated amino acid compound which comprises an amino acid sequence given by SEQ ID NO:2 or SEQ ID NO:4.

24. An isolated nucleic acid compound encoding an amino acid compound of Claim 23.

25. An isolated nucleic acid compound comprising a nucleic acid sequence selected from the group consisting of:

- (a) SEQ ID NO:1 or SEQ ID NO:3;
- (b) nucleotides 25 through 1629 of SEQ ID NO:1 or SEQ ID NO:3;

26. An isolated nucleic acid compound comprising a nucleic acid sequence selected from the group consisting of:

- (a) SEQ ID NO:5; and
- (b) nucleotides 25 through 1629 of SEQ ID NO:5.

27. An isolated nucleic acid compound having a nucleotide sequence which is complementary to the nucleotide sequence given by SEQ ID NO:1 or SEQ ID NO:3.

28. An isolated nucleic acid compound comprising nucleic acid that hybridizes to the complement of the nucleic acid compound that encodes a polypeptide given by SEQ ID NO:2 or SEQ ID NO:4.

29. An expression vector comprising a nucleic acid compound selected from the group consisting of:

- (a) a nucleic acid sequence encoding an amino acid sequence given by SEQ ID NO:2 or SEQ ID NO:4;
- (b) SEQ ID NO:1 or SEQ ID NO:3;
- (c) nucleotides 25 through 1629 of SEQ ID NO:1 or SEQ ID NO:3;
- (d) a nucleotide sequence which is complementary to the nucleotide sequence given by SEQ ID NO:1 or SEQ ID NO:3; and
- (e) a nucleic acid that hybridizes to the complement of the nucleic acid compound that encodes a polypeptide given by SEQ ID NO:2 or SEQ ID NO:4.

30. An expression vector comprising a nucleic acid compound as claimed in Claim 29 in combination with regulatory elements necessary for expression of the nucleic acid compound in a host cell.

31. An expression vector as claimed in Claim 30 for use in a host cell wherein said host cell is a mammalian cell.

32. An expression vector as claimed in Claim 30 for use in a host cell wherein said host cell is a prokaryotic cell.

33. A host cell comprising an expression vector as claimed in Claim 30.

34. A host cell as claimed in Claim 33 wherein the host cell is a mammalian cell.

35. A host cell as claimed in Claim 33 wherein the host cell is a prokaryotic cell.

36. A process for producing an amino acid compound comprising culturing the host cell of Claim 33 under conditions suitable for expression of said amino acid compound and recovering said amino acid compound from the cell culture.

37. An isolated amino acid compound produced by the method of Claim 36.

38. A chimeric molecule comprising an isolated amino acid compound of Claim 24 fused to a heterologous amino acid sequence.

39. An antibody that specifically binds to an amino acid compound as claimed in Claim 24.

40. A composition comprising a therapeutically effective amount of an active agent selected from the group consisting of:

- (a) an amino acid compound as in Claim 24;
- (b) an antibody as in Claim 40; and
- (c) a nucleic acid compound as in Claim 26;

in combination with a pharmaceutically acceptable carrier.

41. A method to identify compounds which inhibit the synthesis or release of beta-amyloid protein, which method comprises:

- (a) isolating an amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4;
- (b) exposing said amino acid sequence to a potential inhibitor of the amino acid sequence;
- (c) introducing a suitable substrate; and
- (d) quantifying the amount of cleavage of the substrate relative to a control in which no potential inhibitor has been introduced.

42. A method of evaluating the effectiveness of a test compound for the treatment or prevention of a condition associated with the synthesis or release of beta-amyloid protein, which method comprises:

- (a) transfecting a mammalian host cell with an expression vector comprising DNA encoding an amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4;
- (b) culturing said host cell under conditions such that said amino acid sequence is expressed;

- (c) exposing said host cell so transfected to a test compound; and
- (d) measuring the change in a physiological condition known to be influenced by said amino acid sequence relative to a control in which the transfected host cell is not exposed to a test compound.

43. A method of treating or preventing neurodegenerative disorders in a patient which comprises administering to a patient in need thereof, an effective amount of an amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4.

44. The method of Claim 43 wherein the neurodegenerative disorder is Alzheimer's disease.--

Remarks

Original claims 1-22 have been canceled. Applicants submit new claims 23-44 to place application in position for allowance. A clean copy of the claims is attached for convenience of review. Applicants assert that each new claim is supported by the specification and that no new matter has been added.

Respectfully submitted,

Paula K. Davis

Paula K. Davis
Agent for Applicants
Registration No. 47,517
Phone: 317-433-3422

Eli Lilly and Company
Patent Division/PKD
Lilly Corporate Center
Indianapolis, Indiana 46285

October 2, 2001

CLAIMS AFTER PRELIMINARY AMENDMENT

23. An isolated amino acid compound which comprises an amino acid sequence given by SEQ ID NO:2 or SEQ ID NO:4.

24. An isolated nucleic acid compound encoding an amino acid compound of Claim 23.

25. An isolated nucleic acid compound comprising a nucleic acid sequence selected from the group consisting of:

- (a) SEQ ID NO:1 or SEQ ID NO:3;
- (b) nucleotides 25 through 1629 of SEQ ID NO:1 or SEQ ID NO:3;

26. An isolated nucleic acid compound comprising a nucleic acid sequence selected from the group consisting of:

- (a) SEQ ID NO:5; and
- (b) nucleotides 25 through 1629 of SEQ ID NO:5.

27. An isolated nucleic acid compound having a nucleotide sequence which is complementary to the nucleotide sequence given by SEQ ID NO:1 or SEQ ID NO:3.

28. An isolated nucleic acid compound comprising nucleic acid that hybridizes to the complement of the nucleic acid compound that encodes a polypeptide given by SEQ ID NO:2 or SEQ ID NO:4.

29. An expression vector comprising a nucleic acid compound selected from the group consisting of:

- (a) a nucleic acid sequence encoding an amino acid sequence given by SEQ ID NO:2 or SEQ ID NO:4;
- (b) SEQ ID NO:1 or SEQ ID NO:3;

- (c) nucleotides 25 through 1629 of SEQ ID NO:1 or SEQ ID NO:3;
- (d) a nucleotide sequence which is complementary to the nucleotide sequence given by SEQ ID NO:1 or SEQ ID NO:3; and
- (e) a nucleic acid that hybridizes to the complement of the nucleic acid compound that encodes a polypeptide given by SEQ ID NO:2 or SEQ ID NO:4.

30. An expression vector comprising a nucleic acid compound as claimed in Claim 29 in combination with regulatory elements necessary for expression of the nucleic acid compound in a host cell.

31. An expression vector as claimed in Claim 30 for use in a host cell wherein said host cell is a mammalian cell.

32. An expression vector as claimed in Claim 30 for use in a host cell wherein said host cell is a prokaryotic cell.

33. A host cell comprising an expression vector as claimed in Claim 30.

34. A host cell as claimed in Claim 33 wherein the host cell is a mammalian cell.

35. A host cell as claimed in Claim 33 wherein the host cell is a prokaryotic cell.

36. A process for producing an amino acid compound comprising culturing the host cell of Claim 33 under conditions suitable for expression of said amino acid compound and recovering said amino acid compound from the cell culture.

37. An isolated amino acid compound produced by the method of Claim 36.

38. A chimeric molecule comprising an isolated amino acid compound of Claim 24 fused to a heterologous amino acid sequence.

39. An antibody that specifically binds to an amino acid compound as claimed in Claim 24.

40. A composition comprising a therapeutically effective amount of an active agent selected from the group consisting of:

- (a) an amino acid compound as in Claim 24;
 - (b) an antibody as in Claim 40; and
 - (c) a nucleic acid compound as in Claim 26;
- in combination with a pharmaceutically acceptable carrier.

41. A method to identify compounds which inhibit the synthesis or release of beta-amyloid protein, which method comprises:

- (a) isolating an amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4;
- (b) exposing said amino acid sequence to a potential inhibitor of the amino acid sequence;
- (c) introducing a suitable substrate; and
- (d) quantifying the amount of cleavage of the substrate relative to a control in which no potential inhibitor has been introduced.

42. A method of evaluating the effectiveness of a test compound for the treatment or prevention of a condition

associated with the synthesis or release of beta-amyloid protein, which method comprises:

- (a) transfecting a mammalian host cell with an expression vector comprising DNA encoding an amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4;
- (b) culturing said host cell under conditions such that said amino acid sequence is expressed;
- (c) exposing said host cell so transfected to a test compound; and
- (d) measuring the change in a physiological condition known to be influenced by said amino acid sequence relative to a control in which the transfected host cell is not exposed to a test compound.

43. A method of treating or preventing neurodegenerative disorders in a patient which comprises administering to a patient in need thereof, an effective amount of an amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4.

44. The method of Claim 43 wherein the neurodegenerative disorder is Alzheimer's disease.



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08 JUL 2002

CERTIFICATE OF MAILING

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ELI LILLY AND COMPANY

By *Hinda M. Durbin*

Date *July 2, 2002*

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	: Gerald Wayne Becker, <i>et al.</i>)	
)	
Serial No.	: 10/018,383)	
)	Confirmation No.:
Filed	: October 30, 2001)	7903
)	
For	: AMYLOID PRECURSOR PROTEASE)	
	PROTEIN AND RELATED)	
	NUCLEIC ACID COMPOUNDS)	
)	
Docket No.	: X-11600)	

SUPPLEMENTAL PRELIMINARY AMENDMENT

Assistant Commissioner for Patents

Arlington, VA 22202

Sir:

In response to the "Notification of Defective Response" dated June 13, 2002, please amend this application as follows:

In the Specification

Delete the Sequence Listing originally filed with the application.

Add the new Sequence Listing as attached.

Remarks

The Sequence Listing for the current application contains unspecified codons in SEQ ID NO:1 from residue 1285 to residue 1365. As originally filed, the CDS for SEQ ID NO:1 was specified, and PatentIn 3.1 automatically translated the polynucleotide into the polypeptide shown in SEQ ID NO:2. Upon this translation, PatentIn automatically translated the unspecified codons (i.e., "nnn") into unspecified proteins (i.e., "Xaa"). Additionally, PatentIn automatically provided the following definition: "The 'Xaa' at location [specified location] stands for Lys, Asn, Arg, Ser, Thr, Ile, Met, Glu, Asp, Gly, Ala, Val, Gln, His, Pro, Leu, **a stop codon**, Tyr, Trp, Cys, or Phe." This definition prompted a "Notification of Defective Response" because of the inclusion of the term "a stop codon." According to a representative of the USPTO, this automatic definition is an error in the PatentIn software and is currently being revised in the program.

The attached Sequence Listing provides a manual definition for all unspecified proteins in SEQ ID NO:2, thereby correcting the automatic definition error generated by the PatentIn software. No new matter has been added by this amendment.

Respectfully submitted,

Paula K. Davis

Paula K. Davis
Agent for Applicants
Registration No. 47,517
Phone: 317-433-3422

Eli Lilly and Company
Patent Division
P.O. Box 6288
Indianapolis, Indiana 46206-6288

02 July 2002

- 1 -

AMYLOID PRECURSOR PROTEIN PROTEASE AND RELATED
NUCLEIC ACID COMPOUNDS

5 This invention relates to proteases which cleave amyloid precursor protein and facilitate β -amyloid peptide release, and accordingly, have utility in identifying compounds which treat or prevent Alzheimer's Disease.

 Alzheimer's disease is a degenerative brain disorder
10 characterized clinically by progressive loss of memory, cognition, reasoning, judgment, and emotional stability that gradually leads to profound mental deterioration and ultimately death. Alzheimer's disease is a very common cause of progressive mental failure (dementia) in aged humans and is believed to represent the fourth most common medical cause of death in the
15 United States. Alzheimer's disease has been observed in races and ethnic groups worldwide and presents a major present and future public health problem. The disease is currently estimated to affect about two to three million individuals in the United States alone. Alzheimer's disease is at present incurable. No treatment that effectively prevents Alzheimer's disease
20 or reverses its symptoms and course is currently known.

 The brains of individuals with Alzheimer's disease exhibit characteristic lesions termed senile (or amyloid) plaques, amyloid angiopathy (amyloid deposits in blood vessels) and neurofibrillary tangles. Large numbers of these lesions, particularly amyloid plaques and neurofibrillary
25 tangles, are generally found in several areas of the human brain important for memory and cognitive function in patients with Alzheimer's disease. Smaller numbers of these lesions in a more restrictive anatomical distribution are also found in the brains of most aged humans who do not have clinical Alzheimer's disease. Amyloid plaques and amyloid angiopathy
30 also characterize the brains of individuals with Trisomy 21 (Down's

- 2 -

syndrome) and Hereditary Cerebral Hemorrhage with Amyloidosis of the Dutch-Type (HCHWA-D).

The principal chemical constituent of the amyloid plaques and vascular amyloid deposits (amyloid angiopathy), characteristic of Alzheimer's disease and the other disorders mentioned above, is an approximately 4.2 kilodalton (kD) protein of about 39-43 amino acids designated the β -amyloid peptide (this protein is also referred to in the literature as β AP, A β , A β P, A-beta, or β /A4). β -Amyloid peptide was first purified and a partial amino acid sequence was provided by Glenner, *et al.*, Biochemical and Biophysical Research Communications, 120:885-890 (1984). The isolation procedure and the sequence data for the first 28 amino acids are described in United States Patent 4,666,829, the entire contents of which are herein incorporated by reference.

Several lines of evidence indicate that progressive cerebral deposition of β -amyloid peptide plays a seminal role in the pathogenesis of Alzheimer's disease and can precede cognitive symptoms by years or decades. See, e.g., D. Selkoe, Neuron, 6:487-498 (1991). An important line of evidence is the discovery that missense DNA mutations at amino acid 717 of the 770-amino acid isoform of amyloid precursor protein can be found in affected members, but not unaffected members, of several families with a genetically determined (familial) form of Alzheimer's disease. Goate, *et al.*, Nature (London), 349:704-706 (1990). Genetic linkage studies have demonstrated that this mutation, as well as certain other mutations in the amyloid precursor protein gene, are the specific molecular cause of Alzheimer's disease in the affected members of such families. In addition, a mutation at amino acid 693 of the 770-amino acid isoform of amyloid precursor protein has been identified as the cause of the β -amyloid peptide deposition disease, and a change from alanine to glycine at amino acid 692 appears to cause a phenotype that resembles Alzheimer's disease in some patients. The discovery of these and other mutations in amyloid precursor protein in

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genetically based cases of Alzheimer's disease support the hypothesis that alteration of amyloid precursor protein and subsequent deposition of its β -amyloid peptide fragment can cause Alzheimer's disease.

5 Molecular biological and protein chemical analyses have shown that the β -amyloid peptide is a small fragment of a much larger precursor protein, the amyloid precursor protein (APP), that is normally produced by cells in many tissues of various animals, including humans. Knowledge of the structure of the gene encoding the amyloid precursor protein has demonstrated that the β -amyloid peptide arises as a peptide fragment that is
10 cleaved from the amyloid precursor protein by proteases.

It is presently believed that a normal (i.e., non-pathogenic) processing of the amyloid precursor protein occurs via cleavage by a putative " α -secretase" which cleaves between amino acids 16 and 17 of the β -amyloid peptide region within the protein. Kang, *et al.*, Nature (London), 325:773-776
15 (1987). It is further believed that pathogenic processing occurs in part via an enzyme designated " β -secretase" which cleaves at the amino-terminus of the β -amyloid peptide region within the precursor protein.

The identification, isolation, and purification of the proteases involved in the processing of amyloid precursor protein would permit
20 chemical modeling of a critical event in the pathology of Alzheimer's disease and would allow the screening of compounds to determine their ability to inhibit formation of β -amyloid peptide.

Despite the progress which has been made in understanding the underlying mechanisms of Alzheimer's disease and other β -amyloid peptide-related diseases, there remains a need to develop methods and compositions
25 for treatment of the diseases. Ideally, the treatment methods would advantageously be based on drugs which are capable of inhibiting β -amyloid peptide release and/or its synthesis in vivo.

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The present invention provides an isolated protein useful as an amyloid precursor protein protease, said compound comprising the amino acid sequence

5								Met	Leu	Arg	Arg	Arg	Gly	Ser	Pro	Gly	
								1					5				
	Met	Gly	Val	His	Val	Gly	Ala	Ala	Leu	Gly	Ala	Leu	Trp	Phe	Cys	Leu	
	10					15					20					25	
10	Thr	Gly	Ala	Leu	Glu	Val	Gln	Val	Pro	Glu	Asp	Pro	Val	Val	Ala	Leu	
					30					35					40		
	Val	Gly	Thr	Asp	Ala	Thr	Leu	Cys	Cys	Ser	Phe	Ser	Pro	Glu	Pro	Gly	
				45					50					55			
15	Phe	Ser	Leu	Ala	Gln	Leu	Asn	Leu	Ile	Trp	Gln	Leu	Thr	Asp	Thr	Lys	
		60						65					70				
	Gln	Leu	Val	His	Ser	Phe	Ala	Glu	Gly	Gln	Asp	Gln	Gly	Ser	Ala	Tyr	
20		75					80					85					
	Ala	Asn	Arg	Thr	Ala	Leu	Phe	Pro	Asp	Leu	Leu	Ala	Gln	Gly	Asn	Ala	
	90				95					100					105		
25	Ser	Leu	Arg	Leu	Gln	Arg	Val	Arg	Val	Ala	Asp	Glu	Gly	Ser	Phe	Thr	
					110					115					120		
	Cys	Phe	Val	Ser	Ile	Arg	Asp	Phe	Gly	Ser	Ala	Ala	Val	Ser	Leu	Gln	
			125						130					135			
30	Val	Ala	Ala	Pro	Tyr	Ser	Lys	Pro	Ser	Met	Thr	Leu	Glu	Pro	Asn	Lys	
		140						145					150				
	Asp	Leu	Arg	Pro	Gly	Asp	Thr	Val	Thr	Ile	Thr	Cys	Ser	Ser	Tyr	Gln	
35		155					160					165					

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	Gly Tyr	Pro Glu	Ala Glu	Val Phe	Trp Gln	Asp Gly	Gln Gly	Val Pro	
	170		175			180			185
5	Leu Thr	Gly Asn	Val Thr	Thr Ser	Gln Met	Ala Asn	Glu Gln	Gly Leu	
		190			195			200	
	Phe Asp	Val His	Ser Ile	Leu Arg	Val Val	Leu Gly	Ala Asn	Gly Thr	
		205			210			215	
10	Tyr Ser	Cys Leu	Val Arg	Asn Pro	Val Leu	Gln Gln	Asp Ala	His Ser	
		220			225			230	
	Ser Val	Thr Ile	Thr Pro	Gln Arg	Ser Pro	Thr Gly	Ala Val	Glu Val	
		235			240			245	
15	Gln Val	Pro Glu	Asp Pro	Val Val	Ala Leu	Val Gly	Thr Asp	Ala Thr	
	250			255		260			265
	Leu Arg	Cys Ser	Phe Ser	Pro Glu	Pro Gly	Phe Ser	Leu Ala	Gln Leu	
20			270			275			280
	Asn Leu	Ile Trp	Gln Leu	Thr Asp	Thr Lys	Gln Leu	Val His	Ser Phe	
		285			290			295	
25	Thr Glu	Gly Arg	Asp Gln	Gly Ser	Ala Tyr	Ala Asn	Arg Thr	Ala Leu	
		300			305			310	
	Phe Pro	Asp Leu	Leu Ala	Gln Gly	Asn Ala	Ser Leu	Arg Leu	Gln Arg	
		315			320			325	
30	Val Arg	Val Ala	Asp Glu	Gly Ser	Phe Thr	Cys Phe	Val Ser	Ile Arg	
	330			335		340			345
	Asp Phe	Gly Ser	Ala Ala	Val Ser	Leu Gln	Val Ala	Ala Pro	Tyr Ser	
35			350			355			360

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Lys Pro Ser Met Thr Leu Glu Pro Asn Lys Asp Leu Arg Pro Gly Asp
365 370 375

Thr Val Thr Ile Thr Cys Ser Ser Tyr Arg Gly Tyr Pro Glu Ala Glu
5 380 385 390

Val Phe Trp Gln Asp Gly Gln Gly Val Pro Leu Thr Gly Asn Val Thr
395 400 405

Thr Ser Gln Met Ala Asn Glu Gln Gly Leu Phe Xaa Xaa Xaa Xaa Xaa
10 410 415 420 425

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
430 435 440

15 Xaa Xaa Xaa Xaa Xaa Xaa Asp Ala His Gly Ser Val Thr Ile Thr Gly
445 450 455

Gln Pro Met Thr Phe Pro Pro Glu Ala Leu Trp Val Thr Val Gly Leu
20 460 465 470

Ser Val Cys Leu Ile Ala Leu Leu Val Ala Leu Ala Phe Val Cys Trp
475 480 485

25 Arg Lys Ile Lys Gln Ser Cys Glu Glu Glu Asn Ala Gly Ala Glu Asp
490 495 500 505

Gln Asp Gly Glu Gly Glu Gly Ser Lys Thr Ala Leu Gln Pro Leu Lys
510 515 520

30 His Ser Asp Ser Lys Glu Asp Asp Gly Gln Glu Ile Ala
525 530 535

hereinafter referred to as SEQ ID NO:2.

35 More preferred is the amyloid precursor protein protease
comprising the amino acid sequence

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Leu Thr Gly Asn Val Thr Thr Ser Gln Met Ala Asn Glu Gln Gly Leu
190 195 200

5 Phe Asp Val His Ser Ile Leu Arg Val Val Leu Gly Ala Asn Gly Thr
205 210 215

Tyr Ser Cys Leu Val Arg Asn Pro Val Leu Gln Gln Asp Ala His Ser
10 220 225 230

Ser Val Thr Ile Thr Pro Gln Arg Ser Pro Thr Gly Ala Val Glu Val
235 240 245

15 Gln Val Pro Glu Asp Pro Val Val Ala Leu Val Gly Thr Asp Ala Thr
250 255 260 265

Leu Arg Cys Ser Phe Ser Pro Glu Pro Gly Phe Ser Leu Ala Gln Leu
270 275 280

20 Asn Leu Ile Trp Gln Leu Thr Asp Thr Lys Gln Leu Val His Ser Phe
285 290 295

Thr Glu Gly Arg Asp Gln Gly Ser Ala Tyr Ala Asn Arg Thr Ala Leu
25 300 305 310

Phe Pro Asp Leu Leu Ala Gln Gly Asn Ala Ser Leu Arg Leu Gln Arg
315 320 325

30 Val Arg Val Ala Asp Glu Gly Ser Phe Thr Cys Phe Val Ser Ile Arg
330 335 340 345

Asp Phe Gly Ser Ala Ala Val Ser Leu Gln Val Ala Ala Pro Tyr Ser
350 355 360

35 Lys Pro Ser Met Thr Leu Glu Pro Asn Lys Asp Leu Arg Pro Gly Asp

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	365	370	375
	Thr Val Thr Ile Thr Cys Ser Ser Tyr Arg Gly Tyr Pro Glu Ala Glu		
	380	385	390
5	Val Phe Trp Gln Asp Gly Gln Gly Val Pro Leu Thr Gly Asn Val Thr		
	395	400	405
	Thr Ser Gln Met Ala Asn Glu Gln Gly Leu Phe Asp Val His Ser Val		
10	410	415	420 425
	Leu Arg Val Val Leu Gly Ala Asn Gly Thr Tyr Ser Cys Leu Val Arg		
	430	435	440
15	Asn Pro Val Leu Gln Gln Asp Ala His Gly Ser Val Thr Ile Thr Gly		
	445	450	455
	Gln Pro Met Thr Phe Pro Pro Glu Ala Leu Trp Val Thr Val Gly Leu		
20	460	465	470
	Ser Val Cys Leu Ile Ala Leu Leu Val Ala Leu Ala Phe Val Cys Trp		
	475	480	485
	Arg Lys Ile Lys Gln Ser Cys Glu Glu Glu Asn Ala Gly Ala Glu Asp		
25	490	495	500 505
	Gln Asp Gly Glu Gly Glu Gly Ser Lys Thr Ala Leu Gln Pro Leu Lys		
	510	515	520
30	His Ser Asp Ser Lys Glu Asp Asp Gly Gln Glu Ile Ala		
	525	530	535

hereinafter referred to as SEQ ID NO:4.

The invention also provides isolated nucleic acid compounds that comprise nucleic acid sequences which encode the amino acid compounds provided. Particularly, the isolated nucleic acid compounds that are provided are preferably DNA, however, nucleic acid compounds which are sense or

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antisense mRNA are also provided. A particularly preferred nucleic acid compound is the DNA compound comprising the sequence

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5  agctgtcagc cgcttcacag gaag atg ctg cgt cgg cgg ggc agc cct ggc 51
    Met Leu Arg Arg Arg Gly Ser Pro Gly
      1                      5

    atg ggt gtg cat gtg ggt gca gcc ctg gga gca ctg tgg ttc tgc ctc 99
    Met Gly Val His Val Gly Ala Ala Leu Gly Ala Leu Trp Phe Cys Leu
10  10                      15                      20                      25

    aca gga gcc ctg gag gtc cag gtc cct gaa gac cca gtg gtg gca ctg 147
    Thr Gly Ala Leu Glu Val Gln Val Pro Glu Asp Pro Val Val Ala Leu
      30                      35                      40

15  gtg ggc acc gat gcc acc ctg tgc tgc tcc ttc tcc cct gag cct ggc 195
    Val Gly Thr Asp Ala Thr Leu Cys Cys Ser Phe Ser Pro Glu Pro Gly
      45                      50                      55

20  ttc agc ctg gca cag ctc aac ctc atc tgg cag ctg aca gat acc aaa 243
    Phe Ser Leu Ala Gln Leu Asn Leu Ile Trp Gln Leu Thr Asp Thr Lys
      60                      65                      70

    cag ctg gtg cac agc ttt gct gag ggc cag gac cag ggc agc gcc tat 291
25  Gln Leu Val His Ser Phe Ala Glu Gly Gln Asp Gln Gly Ser Ala Tyr
      75                      80                      85

    gcc aac cgc acg gcc ctc ttc ccg gac ctg ctg gca cag ggc aac gca 339
    Ala Asn Arg Thr Ala Leu Phe Pro Asp Leu Leu Ala Gln Gly Asn Ala
30  90                      95                      100                      105

    tcc ctg agg ctg cag cgc gtg cgt gtg gcg gac gag ggc agc ttc acc 387
    Ser Leu Arg Leu Gln Arg Val Arg Val Ala Asp Glu Gly Ser Phe Thr
      110                      115                      120

35  tgc ttc gtg agc atc cgg gat ttc ggc agc gct gcc gtc agc ctg cag 435

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	Cys Phe Val Ser Ile Arg Asp Phe Gly Ser Ala Ala Val Ser Leu Gln	
	125	130 135
	gtg gcc gct ccc tac tcg aag ccc agc atg acc ctg gag ccc aac aag	483
5	Val Ala Ala Pro Tyr Ser Lys Pro Ser Met Thr Leu Glu Pro Asn Lys	
	140	145 150
	gac ctg cgg cca ggg gac acg gtg acc atc acg tgc tcc agc tac cag	531
10	Asp Leu Arg Pro Gly Asp Thr Val Thr Ile Thr Cys Ser Ser Tyr Gln	
	155	160 165
	ggc tac cct gag gct gag gtg ttc tgg cag gat ggg cag ggt gtg ccc	579
	Gly Tyr Pro Glu Ala Glu Val Phe Trp Gln Asp Gly Gln Gly Val Pro	
15	170	175 180 185
	ctg act ggc aac gtg acc acg tcg cag atg gcc aac gag cag ggc ttg	627
	Leu Thr Gly Asn Val Thr Thr Ser Gln Met Ala Asn Glu Gln Gly Leu	
	190	195 200
20	ttt gat gtg cac agc atc ctg cgg gtg gtg ctg ggt gca aat ggc acc	675
	Phe Asp Val His Ser Ile Leu Arg Val Val Leu Gly Ala Asn Gly Thr	
	205	210 215
	tac agc tgc ctg gtg cgc aac ccc gtg ctg cag cag gat gcg cac agc	723
25	Tyr Ser Cys Leu Val Arg Asn Pro Val Leu Gln Gln Asp Ala His Ser	
	220	225 230
	tct gtc acc atc aca ccc cag aga agc ccc aca gga gcc gtg gag gtc	771
30	Ser Val Thr Ile Thr Pro Gln Arg Ser Pro Thr Gly Ala Val Glu Val	
	235	240 245
	cag gtc cct gag gac ccg gtg gtg gcc cta gtg ggc acc gat gcc acc	819
	Gln Val Pro Glu Asp Pro Val Val Ala Leu Val Gly Thr Asp Ala Thr	
35	250	255 260 265
	ctg cgc tgc tcc ttc tcc ccc gag cct ggc ttc agc ctg gca cag ctc	867

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	Thr Ser Gln Met Ala Asn Glu Gln Gly Leu Phe Xaa Xaa Xaa Xaa Xaa	
	410	415 420 425
	nnn nnn nnn nnn nnn nnn nnn nnn nnn nnn nnn nnn nnn nnn nnn	1347
5	Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa	
	430	435 440
	nnn nnn nnn nnn nnn nnn gat gcg cac ggc tct gtc acc atc aca ggg	1395
10	Xaa Xaa Xaa Xaa Xaa Xaa Asp Ala His Gly Ser Val Thr Ile Thr Gly	
	445	450 455
	cag cct atg aca ttc ccc cca gag gcc ctg tgg gtg acc gtg ggg ctg	1443
	Gln Pro Met Thr Phe Pro Pro Glu Ala Leu Trp Val Thr Val Gly Leu	
	460	465 470
15	tct gtc tgt ctc att gca ctg ctg gtg gcc ctg gct ttc gtg tgc tgg	1491
	Ser Val Cys Leu Ile Ala Leu Leu Val Ala Leu Ala Phe Val Cys Trp	
	475	480 485
20	aga aag atc aaa cag agc tgt gag gag gag aat gca gga gct gag gac	1539
	Arg Lys Ile Lys Gln Ser Cys Glu Glu Glu Asn Ala Gly Ala Glu Asp	
	490	495 500 505
	cag gat ggg gag gga gaa ggc tcc aag aca gcc ctg cag cct ctg aaa	1587
25	Gln Asp Gly Glu Gly Glu Gly Ser Lys Thr Ala Leu Gln Pro Leu Lys	
	510	515 520
	cac tct gac agc aaa gaa gat gat gga caa gaa ata gcc tga	1629
	His Ser Asp Ser Lys Glu Asp Asp Gly Gln Glu Ile Ala	
30	525	530 535
	ccatgaggac cagggagctg ctacccctcc ctacagctcc tacccctctgg ctgc	1683

hereinafter referred to as SEQ ID NO:1.

35 However, an even more preferred isolated nucleic acid compound is the DNA compound having the sequence

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agctgtcagc cgcctcacag gaag atg ctg cgt cgg cgg ggc agc cct ggc 51
 Met Leu Arg Arg Arg Gly Ser Pro Gly
 1 5

5 atg ggt gtg cat gtg ggt gca gcc ctg gga gca ctg tgg ttc tgc ctc 99
 Met Gly Val His Val Gly Ala Ala Leu Gly Ala Leu Trp Phe Cys Leu
 10 15 20 25

10 aca gga gcc ctg gag gtc cag gtc cct gaa gac cca gtg gtg gca ctg 147
 Thr Gly Ala Leu Glu Val Gln Val Pro Glu Asp Pro Val Val Ala Leu
 30 35 40

15 gtg ggc acc gat gcc acc ctg tgc tgc tcc ttc tcc cct gag cct ggc 195
 Val Gly Thr Asp Ala Thr Leu Cys Cys Ser Phe Ser Pro Glu Pro Gly
 45 50 55

20 ttc agc ctg gca cag ctc aac ctc atc tgg cag ctg aca gat acc aaa 243
 Phe Ser Leu Ala Gln Leu Asn Leu Ile Trp Gln Leu Thr Asp Thr Lys
 60 65 70

25 cag ctg gtg cac agc ttt gct gag ggc cag gac cag ggc agc gcc tat 291
 Gln Leu Val His Ser Phe Ala Glu Gly Gln Asp Gln Gly Ser Ala Tyr
 75 80 85

ggc aac cgc acg gcc ctc ttc ccg gac ctg ctg gca cag ggc aac gca 339
 Ala Asn Arg Thr Ala Leu Phe Pro Asp Leu Leu Ala Gln Gly Asn Ala
 90 95 100

30 tcc ctg agg ctg cag cgc gtg cgt gtg gcg gac gag ggc agc ttc acc 387
 Ser Leu Arg Leu Gln Arg Val Arg Val Ala Asp Glu Gly Ser Phe Thr
 110 115 120

35 tgc ttc gtg agc atc cgg gat ttc ggc agc gct gcc gtc agc ctg cag 435
 Cys Phe Val Ser Ile Arg Asp Phe Gly Ser Ala Ala Val Ser Leu Gln
 125 130 135

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	gtg gcc gct ccc tac tcg aag ccc agc atg acc ctg gag ccc aac aag	483
	Val Ala Ala Pro Tyr Ser Lys Pro Ser Met Thr Leu Glu Pro Asn Lys	
	140 145 150	
5		
	gac ctg cgg cca ggg gac acg gtg acc atc acg tgc tcc agc tac cag	531
	Asp Leu Arg Pro Gly Asp Thr Val Thr Ile Thr Cys Ser Ser Tyr Gln	
	155 160 165	
10		
	ggc tac cct gag gct gag gtg ttc tgg cag gat ggg cag ggt gtg ccc	579
	Gly Tyr Pro Glu Ala Glu Val Phe Trp Gln Asp Gly Gln Gly Val Pro	
	170 175 180 185	
	ctg act ggc aac gtg acc acg tcg cag atg gcc aac gag cag ggc ttg	627
15	Leu Thr Gly Asn Val Thr Thr Ser Gln Met Ala Asn Glu Gln Gly Leu	
	190 195 200	
	ttt gat gtg cac agc atc ctg cgg gtg gtg ctg ggt gca aat ggc acc	675
	Phe Asp Val His Ser Ile Leu Arg Val Val Leu Gly Ala Asn Gly Thr	
20	205 210 215	
	tac agc tgc ctg gtg cgc aac ccc gtg ctg cag cag gat gcg cac agc	723
	Tyr Ser Cys Leu Val Arg Asn Pro Val Leu Gln Gln Asp Ala His Ser	
	220 225 230	
25		
	tct gtc acc atc aca ccc cag aga agc ccc aca gga gcc gtg gag gtc	771
	Ser Val Thr Ile Thr Pro Gln Arg Ser Pro Thr Gly Ala Val Glu Val	
	235 240 245	
	cag gtc cct gag gac ccg gtg gtg gcc cta gtg ggc acc gat gcc acc	819
	Gln Val Pro Glu Asp Pro Val Val Ala Leu Val Gly Thr Asp Ala Thr	
	250 255 260 265	
	ctg cgc tgc tcc ttc tcc ccc gag cct ggc ttc agc ctg gca cag ctc	867
35	Leu Arg Cys Ser Phe Ser Pro Glu Pro Gly Phe Ser Leu Ala Gln Leu	
	270 275 280	

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	aac ctc atc tgg cag ctg aca gac acc aaa cag ctg gtg cac agt ttc	915
	Asn Leu Ile Trp Gln Leu Thr Asp Thr Lys Gln Leu Val His Ser Phe	
	285 290 295	
5	acc gaa ggc cgg gac cag ggc agc gcc tat gcc aac cgc acg gcc ctc	963
	Thr Glu Gly Arg Asp Gln Gly Ser Ala Tyr Ala Asn Arg Thr Ala Leu	
	300 305 310	
10	ttc ccg gac ctg ctg gca caa ggc aat gca tcc ctg agg ctg cag cgc	1011
	Phe Pro Asp Leu Leu Ala Gln Gly Asn Ala Ser Leu Arg Leu Gln Arg	
	315 320 325	
	gtg cgt gtg gcg gac gag ggc agc ttc acc tgc ttc gtg agc atc cgg	1059
15	Val Arg Val Ala Asp Glu Gly Ser Phe Thr Cys Phe Val Ser Ile Arg	
	330 335 340 345	
	gat ttc ggc agc gct gcc gtc agc ctg cag gtg gcc gct ccc tac tcg	1107
	Asp Phe Gly Ser Ala Ala Val Ser Leu Gln Val Ala Ala Pro Tyr Ser	
20	350 355 360	
	aag ccc agc atg acc ctg gag ccc aac aag gac ctg cgg cca ggg gac	1155
	Lys Pro Ser Met Thr Leu Glu Pro Asn Lys Asp Leu Arg Pro Gly Asp	
	365 370 375	
25	acg gtg acc atc acg tgc tcc agc tac cgg ggc tac cct gag gct gag	1203
	Thr Val Thr Ile Thr Cys Ser Ser Tyr Arg Gly Tyr Pro Glu Ala Glu	
	380 385 390	
30	gtg ttc tgg cag gat ggg cag ggt gtg ccc ctg act ggc aac gtg acc	1251
	Val Phe Trp Gln Asp Gly Gln Gly Val Pro Leu Thr Gly Asn Val Thr	
	395 400 405	
	acg tcg cag atg gcc aac gag cag ggc ttg ttt gat gtg cac agc gtc	1299
35	Thr Ser Gln Met Ala Asn Glu Gln Gly Leu Phe Asp Val His Ser Val	
	410 415 420 425	

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	ctg cgg gtg gtg ctg ggt gcg aat ggc acc tac agc tgc ctg gtg cgc	1347
	Leu Arg Val Val Leu Gly Ala Asn Gly Thr Tyr Ser Cys Leu Val Arg	
	430 435 440	
5	aac ccc gtg ctg cag cag gat gcg cac ggc tct gtc acc atc aca ggg	1395
	Asn Pro Val Leu Gln Gln Asp Ala His Gly Ser Val Thr Ile Thr Gly	
	445 450 455	
10	cag cct atg aca ttc ccc cca gag gcc ctg tgg gtg acc gtg ggg ctg	1443
	Gln Pro Met Thr Phe Pro Pro Glu Ala Leu Trp Val Thr Val Gly Leu	
	460 465 470	
	tct gtc tgt ctc att gca ctg ctg gtg gcc ctg gct ttc gtg tgc tgg	1491
15	Ser Val Cys Leu Ile Ala Leu Leu Val Ala Leu Ala Phe Val Cys Trp	
	475 480 485	
	aga aag atc aaa cag agc tgt gag gag gag aat gca gga gct gag gac	1539
	Arg Lys Ile Lys Gln Ser Cys Glu Glu Glu Asn Ala Gly Ala Glu Asp	
20	490 495 500 505	
	cag gat ggg gag gga gaa ggc tcc aag aca gcc ctg cag cct ctg aaa	1587
	Gln Asp Gly Glu Gly Glu Gly Ser Lys Thr Ala Leu Gln Pro Leu Lys	
	510 515 520	
25	cac tct gac agc aaa gaa gat gat gga caa gaa ata gcc tga	1629
	His Ser Asp Ser Lys Glu Asp Asp Gly Gln Glu Ile Ala	
	525 530 535	
30	ccatgaggac caggagctg ctaccctccc ctacagctccc taccctctgg ctgc	1683

hereinafter referred to as SEQ ID NO:3.

Also provided by the present invention are nucleic acid vectors comprising nucleic acids which encode SEQ ID NO:2 or SEQ ID NO:4 or functional equivalents thereof. The preferred nucleic acid vectors are those which are DNA. Most preferred are DNA vectors which comprise the DNA

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sequence which is SEQ ID NO:3. Moreover, DNA vectors of the present invention preferably comprise a promoter positioned to drive expression of said DNA sequence. Those vectors wherein said promoter functions in human embryonic kidney cells (293 cells), AV12 cells, yeast cells or *E.coli* cells are preferred.

The present invention also provides probes and primers useful for molecular biology techniques. A compound which encodes all or part of SEQ ID NO:2 or SEQ ID NO:4 and which is at least 18 consecutive base pairs in length is provided. Preferably, the 18 base pair or more compound is DNA. Most preferred for this use are the DNA compounds which comprise at least 18 consecutive base pairs of SEQ ID NO:1 or SEQ ID NO:3.

Host cells which harbor the nucleic acids provided by the present invention are also provided. A preferred host cell is an oocyte. A preferred oocyte is one which has been injected with sense mRNA or DNA compounds of the present invention. A more preferred oocyte is one which has been injected with sense mRNA or DNA compounds of the present invention in conjunction with DNA or sense mRNA which encodes APP.

Further, this invention provides cells into which the nucleic acid compounds of the present invention may be transfected. Host cells include those which are transfected with a nucleic acid compound which encodes SEQ ID NO:2 or SEQ ID NO:4. Preferred cells include host cells transfected with a DNA vector comprising SEQ ID NO:1 or SEQ ID NO:3. The preferred transfected host cells which encode SEQ ID NO:2 or SEQ ID NO:4 are 293 cells, AV12 cells, yeast cells and *E. coli* cells. Also preferred is a host cell which has been co-transfected with a DNA vector which comprises SEQ ID NO:1 or SEQ ID NO:3 and a DNA vector which comprises the coding sequence of APP. 293 cells, AV12 cells, yeast cells and *E. coli* cells are the preferred co-transfected host cells.

Additionally, the invention provides a method for identifying DNA homologous to a probe of the present invention, which comprises combining test

nucleic acid with the probe under hybridizing conditions and identifying those test nucleic acids which hybridize.

Assays utilizing the compounds provided by the present invention are also provided. The assays provided determine whether a substance is a
5 ligand for β -secretase, said method comprising contacting β -secretase with said substance, monitoring β -secretase activity by physically detectable means, and identifying those substances which interact with or affect β -secretase. Preferred assays of the present invention include a cell culture assay, a high-performance liquid chromatography (HPLC) assay or a synthetic competition assay. Pre-
10 ferred cell culture assays utilize oocytes, AV12, *E. coli*, yeast or 293 cells which co-express nucleic acids which encode β -secretase and APP.

The invention also provides methods for constructing a host cell capable of expressing a nucleic acid compound which encodes an amino acid compound comprising SEQ ID NO:2 or SEQ ID NO:4, said methods comprising
15 transfecting a host cell with a DNA vector comprising a nucleic acid compound encoding SEQ ID NO:2 or SEQ ID NO:4. A preferred method utilizes 293, AV12, yeast or *E. coli* cells as the host cells. A more preferred method includes a DNA vector which comprises SEQ ID NO:1 or SEQ ID NO:3. A most preferred method includes a DNA vector which comprises SEQ ID NO:3. Another preferred
20 method comprises (a) a DNA vector which comprises SEQ ID NO:3 and (b) a DNA expression vector which encodes the APP coding sequence.

Additionally, methods for expressing a nucleic acid sequence which encodes SEQ ID NO:2 or SEQ ID NO:4, or functional equivalents thereof, in a transfected host cell, are also provided. These methods comprise culturing a
25 transfected host cell of the present invention under conditions suitable for gene expression. A preferred method utilizes 293, AV12, yeast or *E. coli* cells as the transfected host cell. A more preferred method utilizes a DNA vector to transfect the host cell. A most preferred method utilizes a DNA vector comprising all or part of SEQ ID NO:3.

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The present invention also provides processes for isolating and purifying an amyloid precursor protein protease, said process comprising: (a) establishing in a suitable medium, a culture of the host cells transformed with a polynucleotide encoding an amyloid precursor protein protease; and (b) isolating said protease from said culture. Compositions comprising a peptide isolated according to such processes are also provided.

In addition, polyclonal and monoclonal antibodies to the peptides of the present invention are also provided.

The terms and abbreviations used in this document have their normal meanings unless otherwise designated. For example "°C" refers to degrees Celsius; "N" refers to normal or normality; "mmol" refers to millimole or millimoles; "g" refers to gram or grams; "ml" means milliliter or milliliters; "M" refers to molar or molarity; "µg" refers to microgram or micrograms; "µl" refers to microliter or microliters; "pU" refers to pico Unit, and the like.

All nucleic acid sequences, unless otherwise designated, are written in the direction from the 5' end to the 3' end, frequently referred to as "5' to 3'".

All amino acid or protein sequences, unless otherwise designated, are written commencing with the amino terminus ("N-terminus") and concluding with the carboxy terminus ("C-terminus").

"Base pair" or "bp" as used herein refers to DNA or RNA. The abbreviations A, C, G, and T correspond to the 5'-monophosphate forms of the deoxyribonucleosides (deoxy)adenosine, (deoxy)cytidine, (deoxy)guanosine, and (deoxy)thymidine, respectively, when they occur in DNA molecules. The abbreviations U, C, G, and A correspond to the 5'-monophosphate forms of the ribonucleosides uridine, cytidine, guanosine, and adenosine, respectively when they occur in RNA molecules. In double stranded DNA, base pair may refer to a partnership of A with T or C with G. In a DNA/RNA, heteroduplex

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base pair may refer to a partnership of A with U or C with G. (See the definition of "complementary", infra.)

5 The terms "digestion" or "restriction" of DNA refers to the catalytic cleavage of the DNA with a restriction enzyme that acts only at certain sequences in the DNA ("sequence-specific endonucleases"). The various restriction enzymes used herein are commercially available and their reaction conditions, cofactors, and other requirements were used as would be known to one of ordinary skill in the art. Appropriate buffers and substrate amounts for particular restriction enzymes are specified by the manufacturer
10 or can be readily found in the literature.

"Ligation" refers to the process of forming phosphodiester bonds between two double stranded nucleic acid fragments. Unless otherwise provided, ligation may be accomplished using known buffers and conditions with a DNA ligase, such as T4 DNA ligase.

15 The term "plasmid" refers to an extrachromosomal (usually) self-replicating genetic element. Plasmids are generally designated by a lower case "p" preceded and/or followed by letters and/or numbers. The starting plasmids herein are either commercially available, publicly available on an unrestricted basis, or can be constructed from available plasmids in
20 accordance with published procedures. In addition, equivalent plasmids to those described are known in the art and will be apparent to the ordinarily skilled artisan.

The term "reading frame" means the nucleotide sequence from which translation occurs "read" in triplets by the translational apparatus of
25 transfer RNA (tRNA) and ribosomes and associated factors, each triplet corresponding to a particular amino acid. To insure against improper translation, the triplet codons corresponding to the desired polypeptide must be aligned in multiples of three from the initiation codon, i.e. the correct "reading frame" being maintained.

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“Recombinant DNA cloning vector” as used herein refers to any autonomously replicating agent, including, but not limited to, plasmids and phages, comprising a DNA molecule to which one or more additional DNA segments can or have been added.

5 The term “recombinant DNA expression vector” as used herein refers to any recombinant DNA cloning vector in which a promoter to control transcription of the inserted DNA has been incorporated.

The term “expression vector system” as used herein refers to a recombinant DNA expression vector in combination with one or more trans-acting factors that specifically influence transcription, stability, or replication of the recombinant DNA expression vector. The trans-acting factor may be expressed from a co-transfected plasmid, virus, or other extrachromosomal element, or may be expressed from a gene integrated within the chromosome.

“Transcription” as used herein refers to the process whereby
15 information contained in a nucleotide sequence of DNA is transferred to a
complementary RNA sequence.

The term “transfection” as used herein refers to the taking up of an expression vector by a host cell whether or not any coding sequences are in fact expressed. Numerous methods of transfection are known to the ordinarily skilled artisan, for example, calcium phosphate co-precipitation, and 20 electroporation. Successful transfection is generally recognized when any indication of the operation of this vector occurs within the host cell.

The term "transformation" as used herein means the introduction of DNA into an organism so that the DNA is replicable, either as an extrachromosomal element or by chromosomal integration. Methods of transforming bacterial and eukaryotic hosts are well known in the art, many of which methods, such as nuclear injection, protoplast fusion or by calcium treatment using calcium chloride are summarized in J. Sambrook, et al., MOLECULAR CLONING: A LABORATORY MANUAL, (1989).

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The term "translation" as used herein refers to the process whereby the genetic information of messenger RNA is used to specify and direct the synthesis of a polypeptide chain.

The term "vector" as used herein refers to a nucleic acid compound used for the transformation of cells in gene manipulation bearing polynucleotide sequences corresponding to appropriate protein molecules which when combined with appropriate control sequences confer specific properties on the host cell to be transformed. Plasmids, viruses, and bacteriophage are suitable vectors. Artificial vectors are constructed by cutting and joining DNA molecules from different sources using restriction enzymes and ligases. The term "vector" as used herein includes Recombinant DNA cloning vectors and Recombinant DNA expression vectors.

The terms "complementary" or "complementarity" as used herein refer to the capacity of purine and pyrimidine nucleotides to associate through hydrogen bonding to form double stranded nucleic acid molecules. The following base pairs are related by complementarity: guanine and cytosine; adenine and thymine; and adenine and uracil. As used herein, "complementary" means that the aforementioned relationship applies to substantially all base pairs comprising two single-stranded nucleic acid molecules over the entire length of said molecules. "Partially complementary" refers to the aforementioned relationship in which one of two single-stranded nucleic acid molecules is shorter in length than the other such that a portion of one of the molecules remains single-stranded.

The term "hybridization" as used herein refers to a process in which a strand of nucleic acid joins with a complementary strand through base pairing. The conditions employed in the hybridization of two completely or nearly completely complementary nucleic acid strands varies with the degree of complementarity of the two strands and the length of the strands. Such techniques and conditions are well known to practitioners in this field.

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"Isolated amino acid sequence" refers to any amino acid sequence, however constructed or synthesized, which is locationally distinct from the naturally occurring sequence.

"Isolated DNA compound" refers to any DNA sequence, however
5 constructed or synthesized, which is locationally distinct from its natural location in genomic DNA.

"Isolated nucleic acid compound" refers to any RNA or DNA sequence, however constructed or synthesized, which is locationally distinct from its natural location.

10 A "primer" is a nucleic acid fragment which functions as an initiating substrate for enzymatic or synthetic elongation.

The term "promoter" refers to a DNA sequence which directs transcription of DNA to RNA.

A "probe" as used herein is a nucleic acid compound or a
15 fragment thereof which hybridizes with a nucleic acid compound which encodes either the entire sequence of SEQ ID NO:2 or SEQ ID NO:4, a sequence complementary to SEQ ID NO:2 or SEQ ID NO:4, or a part thereof.

The term "EST" or "expressed sequence tag" refers to a fragment or sampling of cDNA which encodes a polypeptide of unknown function.

20 The term "stringency" refers to a set of hybridization conditions which may be varied in order to vary the degree of nucleic acid affinity for other nucleic acid. (See the definition of "hybridization", supra.)

The term "antigenically distinct" as used herein refers to a situation in which antibodies raised against an epitope of the proteins of the present invention, or a fragment thereof, may be used to differentiate
25 between the proteins of the present invention and other β -secretase variants. This term may also be employed in the sense that such antibodies may be used to differentiate between the human β -secretase protein and analogous proteins derived from other species.

- 25 -

The term "PCR" as used herein refers to the widely-known polymerase chain reaction employing a thermally-stable polymerase. This technique, as appreciated by those skilled in the art, is employed to amplify a particular nucleic acid fragment.

5 The term “BLAST” as used herein refers to the widely known
basic local alignment search tool. This tool consists of a set of computer-
based programs designed to permit examination of amino acid and nucleic
acid sequence databases for similarity with an isolated sequence of interest.

The term “RACE” refers to the widely known rapid amplification
10 of complimentary ends technique, to amplify and obtain the 5’ and 3’ ends of
isolated cDNA.

Skilled artisans will recognize that the proteins of the present invention can be synthesized by a number of different methods. All of the amino acid compounds of the invention can be made by chemical methods well known in the art, including solid phase peptide synthesis, or recombinant methods. Both methods are described in U.S. Patent 4,617,149, herein incorporated by reference.

The principles of solid phase chemical synthesis of polypeptides are well known in the art and may be found in general texts in the area. See, 20 e.g., H. Dugas and C. Penney, BIOORGANIC CHEMISTRY, (1981) Springer-Verlag, New York, pgs. 54-92. For examples, peptides may be synthesized by solid-phase methodology utilizing an Applied Biosystems 430A peptide synthesizer (commercially available from Applied Biosystems, Foster City California) and synthesis cycles supplied by Applied Biosystems. Protected 25 amino acids, such as *t*-butoxycarbonyl-protected amino acids, and other reagents are commercially available from many chemical supply houses.

Sequential *t*-butoxycarbonyl chemistry using double couple protocols are applied to the starting *p*-methyl benzhydryl amine resins for the production of C-terminal carboxamides. For the production of C-terminal
30 acids, the corresponding pyridine-2-aldoxime methiodide resin is used.

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Asparagine, glutamine, and arginine are coupled using preformed hydroxy benzotriazole esters. The following side chain protection may be used:

Arg, Tosyl

Asp, cyclohexyl

5 Glu, cyclohexyl

Ser, Benzyl

Thr, Benzyl

Tyr, 4-bromo carbobenzoxy

Removal of the *t*-butoxycarbonyl moiety (deprotection) may be
10 accomplished with trifluoroacetic acid (TFA) in methylene chloride.
Following completion of the synthesis the peptides may be deprotected and
cleaved from the resin with anhydrous hydrogen fluoride containing 10%
meta-cresol. Cleavage of the side chain protecting group(s) and of the peptide
from the resin is carried out at zero degrees centigrade or below, preferably -
15 20°C for thirty minutes followed by thirty minutes at 0°C.

After removal of the hydrogen fluoride, the peptide/resin is
washed with ether, and the peptide extracted with glacial acetic acid and
then lyophilized. Purification is accomplished by size-exclusion
chromatography on a Sephadex G-10 (Pharmacia) column in 10% acetic acid.

20 The proteins of the present invention may also be produced by
recombinant methods. Recombinant methods are preferred if a high yield is
desired. A general method for the construction of any desired DNA sequence
is provided in J. Brown, et al., Methods in Enzymology, 68:109 (1979). See
also, J. Sambrook, et al., supra.

25 The basic steps in the recombinant production of desired
proteins are:

- a) construction of a synthetic or semi-synthetic DNA
encoding the protein of interest;

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b) integrating said DNA into an expression vector in a manner suitable for the expression of the protein of interest, either alone or as a fusion protein;

5 c) transforming an appropriate eukaryotic or prokaryotic host cell with said expression vector,

d) culturing said transformed or transfected host cell in a manner to express the protein of interest; and

10

e) recovering and purifying the recombinantly produced protein of interest.

In general, prokaryotes are used for cloning of DNA sequences in constructing the vectors of this invention. Prokaryotes may also be employed in the production of the protein of interest. For example, the Escherichia coli K12 strain 294 (ATCC No. 31446) is particularly useful for the prokaryotic expression of foreign proteins. Other strains of E. coli which may be used (and their relevant genotypes) include the following.

20

Strain	Genotype
DH5a	F ⁻ (j80dlacZDM15), D(lacZYA-argF)U169 supE44, l ⁻ hsdR17(r _K ⁻ , m _K ⁺), recA1, endA1, gyrA96, thi-1, relA1
HB101	supE44, hsdS20(r _B ⁻ m _B ⁻), recA13, ara-14, proA ₂ lacY1, galK2, rpsL20, xyl-5, mtl-1, mcrB, mrr

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JM109	recA1, e14 ⁻ (mcrA), supE44, endA1, hsdR17(r _K ⁻ , m _K ⁺), gyrA96, relA1, thi-1, (lac- proAB), F ⁺ [traD36, proAB+ lacI ^q , lacZ [?] M15]
RR1	supE44, hsdS20(r _B ⁻ m _B ⁻), ara-14 proA ₂ , lacY1, galK2, rpsL20, xyl-5, mtl-5
c1776	F ⁻ , ton, A53, dapD8, minA1, supE42 (glnV42), D(gal-uvrB)40, minB2, rfb-2, gyrA25, thyA142, oms-2, metC65, oms-1, D(bioH-asd)29, cycB2, cycA1, hsdR2, I ⁻
294	endA, thi ⁻ , hsr ⁻ , hsm _K ⁺ (U.S. Patent 4,366,246)
LE392	F ⁻ , hsdR514 (r ⁻ m ⁻), supE44, supF58, lacY1, or Dlac(I-Y)6, galK2, glaT22, metB1, trpR55, I ⁻

These strains are all commercially available from suppliers such as: Bethesda Research Laboratories, Gaithersburg, Maryland 20877 and Stratagene Cloning Systems, La Jolla, California 92037; or are readily available to the public from sources such as the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland, 10852-1776. Except where otherwise noted, these bacterial strains can be used interchangeably. The genotypes listed are illustrative of many of the desired characteristics for choosing a bacterial host and are not meant to limit the invention in any way. The genotype designations are in accordance with standard nomenclature. See, for example, J. Sambrook, et al., supra.

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In addition to the strains of E. coli discussed supra, bacilli such as Bacillus subtilis, other enterobacteriaceae such as Salmonella typhimurium or Serratia marcescans, and various Pseudomonas species may be used. In addition to these gram-negative bacteria, other bacteria, especially Streptomyces, spp., may be employed in the prokaryotic cloning and expression of the proteins of this invention.

Promoters suitable for use with prokaryotic hosts include the b-lactamase [vector pGX2907 (ATCC 39344) contains the replicon and b-lactamase gene] and lactose promoter systems [Chang et al., Nature (London), 275:615 (1978); and Goeddel et al., Nature (London), 281:544 (1979)], alkaline phosphatase, the tryptophan (trp) promoter system [vector pATH1 (ATCC 37695) is designed to facilitate expression of an open reading frame as a trpE fusion protein under control of the trp promoter] and hybrid promoters such as the tac promoter (isolatable from plasmid pDR540 ATCC-37282). However, other functional bacterial promoters, whose nucleotide sequences are generally known, enable one of skill in the art to ligate them to DNA encoding the proteins of the instant invention using linkers or adapters to supply any required restriction sites. Promoters for use in bacterial systems also will contain a Shine-Dalgarno sequence operably linked to the DNA encoding the desired polypeptides. These examples are illustrative rather than limiting.

The proteins of this invention may be synthesized either by direct expression or as a fusion protein comprising the protein of interest as a translational fusion with another protein or peptide which may be removable by enzymatic or chemical cleavage. It is often observed in the production of certain peptides in recombinant systems that expression as a fusion protein prolongs the lifespan, increases the yield of the desired peptide, or provides a convenient means of purifying the protein of interest. A variety of peptidases (e.g. trypsin) which cleave a polypeptide at specific sites or digest the peptides from the amino or carboxy termini (e.g. diaminopeptidase) of the peptide

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chain are known. Furthermore, particular chemicals (e.g. cyanogen bromide) will cleave a polypeptide chain at specific sites. The skilled artisan will appreciate the modifications necessary to the amino acid sequence (and synthetic or semi-synthetic coding sequence if recombinant means are employed) to incorporate site-specific internal cleavage sites. See e.g., P. Carter, "Site Specific Proteolysis of Fusion Proteins", Chapter 13 in PROTEIN PURIFICATION: FROM MOLECULAR MECHANISMS TO LARGE SCALE PROCESSES, American Chemical Society, Washington, DC (1990).

In addition to cloning and expressing the genes of interest in the prokaryotic systems discussed above, the proteins of the present invention may also be produced in eukaryotic systems. The present invention is not limited to use in a particular eukaryotic host cell. A variety of eukaryotic host cells are available from depositories such as the American Type Culture Collection (ATCC) and are suitable for use with the vectors of the present invention. The choice of a particular host cell depends to some extent on the particular expression vector used to drive expression of the amyloid precursor protein protease-encoding nucleic acids of the present invention. Exemplary host cells suitable for use in the present invention are listed in Table I. Theses exemplary host cells are merely illustrative of the many eukaryotic cells available for use with the present invention and are not meant in any way to limit the scope of the present invention.

Table I

Host Cell	Origin	Source
HepG-2	Human Liver Hepatoblastoma	ATCC HB 8065
CV-1	African Green Monkey Kidney	ATCC CCL 70
LLC-MK ₂	Rhesus Monkey Kidney	ATCC CCL 7
3T3	Mouse Embryo Fibroblasts	ATCC CCL 92
CHO-K1	Chinese Hamster Ovary	ATCC CCL 61
HeLa	Human Cervix Epitheloid	ATCC CCL 2

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RPMI8226	Human Myeloma	ATCC CCL 155
H4IIEC3	Rat Hepatoma	ATCC CCL 1600
C1271	Mouse Fibroblast	ATCC CCL 1616
293	Human Embryonal Kidney	ATCC CRL 1573
Sf9	Fall armyworm ovary <u>Spodoptera</u>	ATCC CRL-1711
	<u>frugiperda</u>	
HS-Sultan	Human Plasma Cell Plasmocytoma	ATCC CCL 1484
BHK-21	Baby Hamster Kidney	ATCC CCL 10

A preferred cell line employed in the expression of the protein of the present invention is the widely available 293 cell line. As noted, this cell line was constructed from human embryonal kidney tissue and is available from American Type Culture Collection under the accession number ATCC CCL 1573.

A wide variety of vectors, some of which are discussed below, exists for the transformation of mammalian host cells, but the specific vectors described herein are merely illustrative and are in no way intended to limit the scope of the present invention.

Some illustrative vectors include the pSV2-type vectors which comprise segments of the simian virus 40 (SV40) genome that constitute a defined eukaryotic transcription unit-promoter, intervening sequence, and polyadenylation site. In the absence of the SV40 T antigen, the plasmid pSV2-type vectors transform mammalian and other eukaryotic host cells by integrating into the host cell chromosomal DNA. A large number of plasmid pSV2-type vectors have been constructed, such as plasmid pSV2-gpt, pSV2-neo, pSV2-dhfr, pSV2-hyg, and pSV2-b-globin, in which the SV40 promoter drives transcription of an inserted gene. These vectors are suitable for use with the coding sequences of the present invention and are widely available

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from sources such as the ATCC or the Northern Regional Research Laboratory (NRRL), 1815 N. University Street, Peoria, Illinois, 61604.

The plasmid pSV2-dhfr (ATCC 37146) comprises a murine dihydrofolate reductase (dhfr) gene under the control of the SV40 early promoter. Under the appropriate conditions, the dhfr gene is known to be amplified, or copied, in the host chromosome. This amplification can result in the amplification of closely-associated DNA sequences and can, therefore, be used to increase production of a protein of interest. See, e.g., J. Schimke, Cell, 35:705-713 (1984).

Plasmids constructed for expression of the proteins of the present invention in mammalian and other eukaryotic host cells can utilize a wide variety of promoters. The present invention is in no way limited to the use of the particular promoters exemplified herein. Promoters such as the SV40 late promoter, promoters from eukaryotic genes, such as, for example, the estrogen-inducible chicken ovalbumin gene, the interferon genes, the gluco-corticoid-inducible tyrosine aminotransferase gene, and the thymidine kinase gene, and the major early and late adenovirus genes can be readily isolated and modified to express the genes of the present invention. Eukaryotic promoters can also be used in tandem to drive expression of a coding sequence of this invention. Furthermore, a large number of retroviruses are known that infect a wide range of eukaryotic host cells. The long terminal repeats in the retroviral DNA frequently encode functional promoters and, therefore, may be used to drive expression of the nucleic acids of the present invention.

Plasmid pRSVcat (ATCC 37152) comprises portions of a long terminal repeat of the Rous Sarcoma virus, a virus known to infect chickens and other host cells. This long terminal repeat contains a promoter which is suitable for use in the vectors of this invention. H. Gorman, et al., Proceedings of the National Academy of Sciences (USA), 79:6777 (1982). The plasmid pMSVi (NRRL B-15929) comprises the long terminal repeats of the

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Murine Sarcoma virus, a virus known to infect mouse and other host cells. The mouse metallothionein promoter has also been well characterized for use in eukaryotic host cells and is suitable for use in the expression of the nucleic acids of the present invention. The mouse metallothionein promoter is
5 present in the plasmid pDBPV-MMTneo (ATCC 37224) which can serve as the starting material of other plasmids of the present invention.

Another useful expression vector system employs one of a series of vectors containing the BK enhancer, an enhancer derived from the BK virus, a human papovavirus. The most preferred such vector systems are
10 those which employ not only the BK enhancer but also the adenovirus-2-early region 1A (E1A) gene product. The E1A gene product (actually, the E1A gene produces two products, which are collectively referred to herein as "the E1A gene product") is an immediate-early gene product of adenovirus, a large DNA virus.

15 An additional useful eukaryotic expression vector is the phd series of vectors which comprise a BK enhancer in tandem with the adenovirus late promoter to drive expression of useful products in eukaryotic host cells. The construction and method of using the phd plasmid, as well as related plasmids, are described in U.S. Patents 5,242,688, issued September
20 7, 1993, and 4,992,373, issued February 12, 1991, all of which are herein incorporated by reference. Escherichia coli K12 GM48 cells harboring the plasmid phd are available as part of the permanent stock collection of the Northern Regional Research Laboratory under accession number NRRL B-18525. The plasmid may be isolated from this culture using standard
25 techniques.

The plasmid phd contains a unique BclI site which may be utilized for the insertion of the gene encoding the protein of interest. The skilled artisan understands that linkers or adapters may be employed in cloning the gene of interest into this BclI site. The phd series of plasmids
30 functions most efficiently when introduced into a host cell which produces the

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E1A gene product, cell lines such as AV12-664, 293 cells, and others, described supra.

Transformation of the mammalian cells can be performed by any of the known processes including, but not limited to, the protoplast fusion method, the calcium phosphate co-precipitation method, electroporation and the like. See, e.g., J. Sambrook, et al., supra, at 3:16.30-3:16.66.

Other routes of production are well known to skilled artisans. In addition to the plasmid discussed above, it is well known in the art that some viruses are also appropriate vectors. For example, the adenovirus, the adeno-associated virus, the vaccinia virus, the herpes virus, the baculovirus, and the rous sarcoma virus are useful. Such a method is described in U.S. Patent 4,775,624, herein incorporated by reference. Several alternate methods of expression are described in J. Sambrook, et al., supra, at 16.3-17.44.

In addition to prokaryotes and mammalian host cells, eukaryotic microbes such as yeast cultures may also be used. The imperfect fungus Saccharomyces cerevisiae, or common baker's yeast, is the most commonly used eukaryotic microorganism, although a number of other strains are commonly available. For expression in Saccharomyces sp., the plasmid YRp7 (ATCC-40053), for example, is commonly used. See, e.g., L. Stinchcomb, et al., Nature (London), 282:39 (1979); J. Kingsman et al., Gene, 7:141 (1979); S. Tschemper et al., Gene, 10:157 (1980). This plasmid already contains the trp gene which provides a selectable marker for a mutant strain of yeast lacking the ability to grow in tryptophan.

Suitable promoting sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase [found on plasmid pAP12BD (ATCC 53231) and described in U.S. Patent No. 4,935,350, issued June 19, 1990, herein incorporated by reference] or other glycolytic enzymes such as enolase [found on plasmid pAC1 (ATCC 39532)], glyceraldehyde-3-phosphate dehydrogenase [derived from plasmid pHcGAPC1 (ATCC 57090, 57091)], hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-

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phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase, as well as the alcohol dehydrogenase and pyruvate decarboxylase genes of Zymomonas mobilis (United States Patent No. 5,000,000 issued March 19, 1991, herein incorporated by reference).

Other yeast promoters, which are inducible promoters, having the additional advantage of their transcription being controllable by varying growth conditions, are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, metallothionein [contained on plasmid vector pCL28XhoLHBPV (ATCC 39475) and described in United States Patent No. 4,840,896, herein incorporated by reference], glyceraldehyde 3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose [e.g. GAL1 found on plasmid pRY121 (ATCC 37658)] utilization. Suitable vectors and promoters for use in yeast expression are further described in R. Hitzeman *et al.*, European Patent Publication No. 73,657A. Yeast enhancers such as the UAS Gal from Saccharomyces cerevisiae (found in conjunction with the CYC1 promoter on plasmid YEpsc--h11beta ATCC 67024), also are advantageously used with yeast promoters.

Practitioners of this invention realize that, in addition to the above-mentioned expression systems, cloned cDNA may also be employed in the production of transgenic animals in which a test mammal, usually a mouse, in which expression or overexpression of the proteins of the present invention can be assessed. The nucleic acids of the present invention may also be employed in the construction of "knockout" animals in which the expression of the native cognate of the gene is suppressed.

Skilled artisans will recognize that the protease of the present invention, as well as the nucleic acid compounds encoding said protease, can be isolated and purified from cultured host cells transiently expressing β -secretase activity. The following examples further illustrate such isolation

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and purification processes, however, these examples are not in any way to be interpreted as limiting the scope of the present invention.

Isolation and Purification of β -secretase

5

Example 1

293 Cell Source

293 Cells were obtained as by-products from 25- to 30-day fermentations at Lilly Technology Center. (Eli Lilly and Co., Indianapolis, IN.)

10

Example 2

β -Secretase Activity Assay

The enzymatic activity of beta-secretase was determined by an ELISA assay, modified in form over an assay appearing in application No. PCT/US96/09985, the entire content of which is herein incorporated by reference. Using MBP-APPc125sw as the substrate, the activity was determined by measuring the MBP-APPc26sw cleavage product with a polyclonal antibody, 192sw, highly specific to the product. MBP-APPc125sw is a fusion substrate in which maltose binding protein is fused to the 125-residue C-terminal portion of the swedish amyloid precursor protein. For the enzymatic activity analysis, one picounit (pU) is defined as the amount of the enzyme that catalyzes the conversion of MBP-APP125sw to one picomole of MBP-APPc26sw per ml in 2-hr under the optimized reaction conditions of the Elisa assay (see Table II, infra). To calculate the enzyme specific activity, the protein concentration was first determined using the well known Bio-Rad microassay method. (Rio-Rad Laboratories, Hercules, CA, 94547)

Example 3

β-Secretase Purification

The purification of beta-secretase was carried out by a protocol modified over the purification methods appearing in application No. PCT/US96/09985, the entire content of which is herein incorporated by reference. The enzyme purification protocol was undertaken at 4 C. β-Secretase activity and protein recovery data are provided in Table III, infra.

Step 1:

Membrane Extract: About 400-g of 293 cells were resuspended in 2,000-ml of 20 mM HEPES (Sigma Chemical Co., St Louis, MO), pH 7.5, 2 mM EDTA (Sigma Chemical Co., St Louis, MO) and 0.25% sucrose (Buffer 1A). The cell suspension was homogenized in an 1000-ml aliquot and the cells were broken with an Brinkmann Homogenizer (POLYTRON, Switzerland) according to the following conditions: at setting 4 for 2- and 1-min; and at setting 5 for 1- and 0.5-min (with 3-min cooling time after each homogenization). The broken cell suspension was centrifuged at 1,000xg for 20-min; the resulting supernatant (post-nuclear supernatant; PNS1) was saved. The pellet was resuspended in 1,200-ml of Buffer 1A, homogenized, broken and centrifuged as described above; the supernatant (PNS2) was saved and the pellet was discarded. PNS1 and PNS2 were combined (2,700-ml) and centrifuged at 45,000xg for 1-hr; the resulting pellet (P2; containing membranes) was saved and the supernatant was discarded. This pellet was resuspended in 3,200-ml of 20 mM MES (Sigma Chemical Co., St Louis, MO), pH 6.0, 2 mM EDTA, 0.5% Triton X-100, 150 mM NaCl, 0.2 mM AEBSF, 5 mg/ml leupeptin, 2 mg/ml E64 and 1 mg/ml pepstatin A (Buffer 1B); all four protease inhibitors were from Calbiochem; San Diego, CA). The suspension was stirred continuously for 1-hr for extraction of beta-secretase and centrifuged at

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16,000xg for 1-hr; the pellet was discarded. The resulting supernatant (3,120-ml) was adjusted to pH 7.5 with 1 M Tris base and filtered through 0.45-mm Zapcap-S (Schleicher & Schuell; Keene, NH); the filtrate was the P2 membrane extract (P2ME, 3,170-ml).

5

A second P2ME was prepared similarly from another 400g of 293 cells. The combined beta-secretase activity and protein data for the P2ME fractions are shown in Table III. However, beta-secretase activity from each P2ME was purified separately in the next step.

10

Step 2:

Wheat Germ Agglutinin (WGA) Agarose Eluate: The first P2ME was loaded to a WGA-Agarose column (Vector Lab.; Burlingame, CA) (1000-ml), pre-equilibrated with 1500-ml of 20 mM Tris, pH 7.5, 2 mM EDTA, 0.5% Triton X-100 and 150 mM NaCl (Buffer 2A). The WGA column, which binds to N-acetyl-D-glucosamine glycopeptides, was washed with 1000-ml of Buffer 2A and bound proteins were eluted with a linear gradient of 0-10% chitin hydrolysate (Vector Lab.) in a total volume of 900-ml constructed from Buffer 2A and Buffer 2B (20 mM Tris, pH 7.5, 2 mM EDTA, 0.5% Triton X-100 and 10% chitin hydrolysate). The fractions containing higher specific activities of beta-secretase were pooled as the WGA-eluate (180-ml).

15

The WGA-Agarose column was regenerated and equilibrated. The second WGA-eluate (170-ml) was obtained similarly from the second P2ME. The two WGA-eluates were combined.(see Table III). In addition, the two WGA-Agarose washings were combined.(see Table III). The WGA-eluate and the WGA-wash were purified together in the next step.

25

DEAE Eluate. S-200-eluates 1 and 2a were de-salted with a Sepharose G-25 column (100-ml; Amersham Pharmacia Biotech) and S-200-eluate 2b was diluted 6x with Buffer 7A (same as 5A) to 25 mM NaCl. To the resulting S-200-eluates; 200-, 200- and 100 µl of DEAE-Sepharose was added, respectively. For effective binding of the activity to the resin, the three samples were agitated continuously overnight. The samples were centrifuged at 10,000xg for 10-min to collect DEAE-Sepharose beads. The bound proteins

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were eluted from DEAE-Sepharose beads of the three samples with, respectively, 2x200-, 2x400- and 2x100 µl of Buffer 7B (Buffer 7A plus 1 M NaCl). The three DEAE-eluates (400-, 800-, and 200 µl; Table III) were treated separately in the next step.

5

Step 8:

De-Glycosylation. To each of the three DEAE-eluates, beta-mercaptoethanol (reducing agent) was added to a final concentration of 3 mM. PNGase F (100 mU/40-ml from Glyko, Novato, CA); an N-linked de-glycosylation enzyme, was added as three aliquots to each sample to a final ratio of about 1:4 (V/V). After each PNGase addition, the samples were placed in a 37 °C incubator for one day. After a total incubation time of three days, beta-secretase activity was stable in two samples and there was a slight (about 20%) activity loss in the other sample (Table III). The three samples (De-Gly) were purified separately in the next and last step.

15

Step 9:

Mini Q Eluate. Each of the three De-Gly samples was de-salted with a Sephadex PD-10 column (Amersham Pharmacia Biotech). Desalted De-Gly sample 1 was loaded to a Mini Q PE column (0.8-ml from Amersham Pharmacia Biotech), a high resolving anion exchanger, pre-equilibrated with Buffer 9 A (same as Buffer 7A). The bound proteins were washed and eluted with a step-wise NaCl gradient in Buffer 7A in a total volume of 35.6-ml as shown below: 7.6-ml at 0 mM NaCl, 16-ml from 0 to 250 mM, 8-ml from 250 to 500 mM and 4-ml at 500 mM. Two activity peaks were observed. Desalted De-Gly 2a were purified in a similar way; two activity peaks were also observed. Desalted De-Gly 2b sample was purified with a Mini Q PC column (0.24-ml from Amersham Pharmacia Biotech) using a step-wise NaCl gradient in Buffer 7A in a total volume of 13.2-ml (4.8-ml at 0 mM NaCl, 4.8-ml from 0 to 250 mM, 2.4-ml from 250 to 500 mM and 1.2-ml at 500 mM).

25

30

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Only one major activity peak was observed. The resulting beta-secretase activity and protein data are shown in Table III.

Beta-secretase activity was purified up to 3,000-fold to apparent homogeneity by the 9-step procedure. Note, however, that the actual activity
 5 enrichment was likely much greater excluding Triton X-100 interference to the low level protein determination in Step 9 above.

Table II
 Optimized Assay for β -Secretase Activity

Assay Parameter	Optimized Activity Conditions
Enzyme*	0.015-0.095 pU for Linear Response (With 0.02 to 0.06 % Triton X-100)
Substrate	~60 nM for Maximal Activity (With ~20 mM Guanidine HCl)
pH	5.25 to 5.75 for Maximal Activity
Reaction Time	≥ 4 hours for Linear Response
Kinetic Constants	
K_m	300 nM
V_{max}	2 nmol/hr/mg protein

*Using 0.313- μ l of Mini Q-Eluate 2a .

Note: The enzymatic reaction was carried out at room temperature.
 The purified enzyme was stable at 4 or -20 C for at least two weeks.

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Table III

	Fraction (step)	Total Activity (pU)	Total Protein (mg)	Sp. Act. (pU/mg)	Recovery (%)
5	P2ME (1)	35,038	23,245	1.507	100
	WGA-Wash (2)	2,160	206	10.49	6
10	WGA-E* (2)	8,330	652	12.78	24
	HiQ-E* (3)	5,998	259	23.16	17
	HA-Wash (4)	2,550	20.4	125.0	7.3
15	Mono Q-E*1 (5)	1,248	3.99	312.7	3.6
	Mono Q-E*2 (5)	2,240	6.43	348.4	6.4
	S-200-E*1 (6)	199.5	0.90	221.7	0.6
20	S-200-E*2b (6)	386.5	1.54	251.0	1.1
	S-200-E*2c (6)	60.0	0.16	375.0	0.2
	DEAE-E*1 (7)	239.3	0.406	574.5	0.7
	DEAE-E*2 (7)	771.8	0.642	1202	2.2
25	DEAE-E*3 (7)	8.35	0.048	174.0	---
	De-Gly*1 (8)	222.5	0.397	560.5	0.6
	De-Gly*2 (8)	534.3	0.634	842.7	1.5
	De-Gly*3 (8)	11.33	0.046	246.2	---
30	Mini Q-E*1a (9)	121.8	0.049	2485	0.3
	Mini Q-E*1b (9)	76.8	0.045	1706	0.2
	Mini Q-E*2a (9)	247.0**	0.087	2839	0.7
	Mini Q-E*2b (9)	369.8**	0.121	3056	1.1
35	Mini Q-E*3 (9)	2.95	0.009	327.8	---

*E = Eluate; FT = Flow-Through; De-Gly = De-Glycosylated

** Mini Q-E 1a and Mini Q-E 1b each contained two activity peaks.

- 5 Mini Q-Eluate 2a represents the first peak from fraction Q-E 1a combined with the first peak from fraction

Q-E 1b.

Mini Q-Eluate 2b represents the second peak from fraction Q-E 1a combined with the second peak from fraction Q-E 1b.

10

- Skilled artisans will recognize that some alterations of SEQ ID NO:2 or SEQ ID NO:4 will fail to change the function of the amino acid compound. For instance, some hydrophobic amino acids may be exchanged for other hydrophobic amino acids. Those altered amino acid compounds
- 15 which confer substantially the same function in substantially the same manner as the exemplified amino acid compounds are also encompassed within the present invention. Typical such conservative substitutions attempt to preserve the: (a) secondary or tertiary structure of the polypeptide backbone; (b) the charge or hydrophobicity of the residue; or (c) the bulk of
- 20 the side chain. Some examples of such conservative substitutions of amino acids, resulting in the production of proteins which are functional equivalents of the protein of SEQ ID NO:2 or SEQ ID NO:4 are shown in Table II, infra.

Table IV

	Original Residue	Exemplary Substitutions
	Ala	Ser, Gly
	Arg	Lys
5	Asn	Gln, His
	Asp	Glu
	Cys	Ser
	Gln	Asn
	Glu	Asp
10	Gly	Pro, Ala
	His	Asn, Gln
	Ile	Leu, Val
	Leu	Ile, Val
	Lys	Arg, Gln, Glu
15	Mel	Leu, Ile
	Phe	Met, Leu, Gyr
	Ser	Thr
	Thr	Ser
	Trp	Tyr
20	Tyr	Trp, Phe
	Val	Ile, Leu

These substitutions may be introduced into the protein in a variety of ways, such as during the chemical synthesis or by chemical modification of an amino acid side chain after the protein has been prepared.

Alterations of the protein having a sequence which corresponds to the sequence of SEQ ID NO:2 or SEQ ID NO:4 may also be induced by alterations of the nucleic acid compounds which encodes these proteins. These mutations of the nucleic acid compound may be generated by either

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random mutagenesis techniques, such as those techniques employing chemical mutagens, or by site-specific mutagenesis employing oligonucleotides. In addition, allelic variants of the gene encoding the protein of the present invention may also be purified by the processes of the present invention. Those nucleic acid compounds which confer substantially the same function in substantially the same manner as the exemplified nucleic acid compounds are also encompassed within the present invention.

Other embodiments of the present invention include nucleic acid compounds which comprise isolated nucleic acid sequences which encode SEQ ID NO:2 or SEQ ID NO:4. As skilled artisans will recognize, the amino acid compounds of the invention can be encoded by a multitude of different nucleic acid sequences because most of the amino acids are encoded by more than one nucleic acid triplet due to the degeneracy of the amino acid code. Because these alternative nucleic acid sequences would encode the same amino acid sequences, the present invention further comprises these alternate nucleic acid sequences.

The full length nucleic acid clones which encode the amino acid sequences given by SEQ ID NO:2 and SEQ ID NO:4, where determined using standard methods, essentially as follows. Fractions of purified beta-secretase, as isolated by the methods described in Examples 1 through 3 above, were applied to an SDS-polyacrylamide gel. Following electrophoresis, the proteins were transferred electrophoretically to a polyvinylidene difluoride (PVDF) membrane and stained with coomassie blue. The stained protein bands were then excised from the membrane and placed in an automatic protein sequencer where the amino acid sequence of the protein was determined using widely known automated Edman chemistry. N-terminal fragments of the determined protein sequence, of approximately 17-20 amino acids, were then searched against protein sequence databases using commercially available BLAST methodology. A BLAST analysis of the proprietary INCYTE database (INCYTE Pharmaceuticals, Palo Alto, CA,

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94304) revealed a series of ESTs showing homology to the determined experimental sequence. The ESTs were subsequently assembled into a contiguous cDNA sequence.

The transcript of the assembled ESTs, however, did not contain
5 a starting methionine. In order to elongate the 5' end of the experimental sequence 5' RACE (rapid amplification of complimentary ends) was performed. [see generally: RACE, as described in M.A. Fohrman, PCR
Protocols: A Guide to Methods and Applications, McInnis et al., Eds.,
Academic Press, Inc, San Diego, CA, pp. 28-29 (1990); see also U.S. Patent
10 No. 5,470,722; and also see Ausubel et al, Current Protocols in Molecular Biology, Ch. 15, Eds., Wiley and Sons, N.Y. (1989-1990)] After designing suitable primers based on the information contained in the EST contiguous cDNA sequence, RACE-ready cDNA (CLONTECH Laboratories, Palo Alto, CA, 94303) was used as a template to elongate and amplify the 5' end until a
15 starting methionine was detected. PCR was then employed to generate the full length cDNA clone. E. coli were subsequently transformed with the full length cDNAs comprising SEQ ID NO:1 and SEQ ID NO:3 in order to generate sufficient copies of the nucleic acids for sequence determination.

The gene encoding the amyloid precursor protein protease of the
20 present invention may also be produced using synthetic methodology, which synthesis is well known in the art. See, e.g., E.L. Brown, R. Belagaje, M.J. Ryan, and H.G. Khorana, Methods in Enzymology, 68:109-151 (1979). Additionally, the nucleic acid sequences corresponding to the gene encoding the amyloid precursor protein protease of the present invention, can also be
25 generated using conventional DNA synthesizing apparatuses such as the Applied Biosystems Model 380A or 380B DNA synthesizers. (commercially available from Applied Biosystems, Inc., 850 Lincoln Center Drive, Foster City, CA 94404) These synthesizers employ phosphoramidite chemistry. In the alternative, the more traditional phosphotriester chemistry may be

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employed to synthesize the nucleic acids of this invention. See, e.g., M.J. Gait, ed., OLIGONUCLEOTIDE SYNTHESIS, A PRACTICAL APPROACH, (1984).

The synthetic amyloid precursor protein protease gene may be designed to possess restriction endonuclease cleavage sites at either end of the transcript to facilitate isolation from and integration into expression and amplification plasmids. The restriction sites are chosen so as to properly orient the coding sequence of the target enzyme with control sequences to achieve proper in-frame reading and expression of the amyloid precursor protein protease molecule. A variety of other such cleavage sites may be incorporated depending on the particular plasmid constructs employed and may be generated by techniques well known in the art.

In an alternative methodology, the desired DNA sequences can be generated using the polymerase chain reaction as described in U.S. Patent No. 4,889,818, which is herein incorporated by reference.

In addition to the deoxyribonucleic acid compounds described supra, the present invention also encompasses ribonucleic acid compounds which have a sequence which encodes SEQ ID NO:2 or SEQ ID NO:4. Particularly, the present invention provides the RNA sequence represented by

20 GCUGUCAGC CGCCUCACAG GAAGAUGCUG. CGUCGGCGGG GCAGCCCUGG 50
CAUGGGUGUG CAUGUGGGUG CAGCCCUGGG AGCACUGUGG UUCUGCCUCA 100
25 CAGGAGCCCU GGAGGUCCAG GUCCCUAAG ACCCAGUGGU GGCACUGGUG 150
GGCACCGAUG CCACCCUGUG CUGCUCUUC UCCCCUGAGC CUGGCUUCAG 200
CCUGGCACAG CUCAACCUCA UCUGGCAGCU GACAGAUACC AAACAGCUGG 250
30 UGCACAGCUU UGCUGAGGGC CAGGACCAGG GCAGCGCCUA UGCCAACCGC 300
ACGGCCCUUC UCCCGGACCU GCUGGCACAG GGCAACGCAU CCCUGAGGCU 350

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UGCGGGUGGU GCUGGGUGCG AAUGGCACCU ACAGCUGCCU GGUGCGCAAC 1400
 CCCGUGCUGC AGCAGGAUGC GCACGGCUCU GUCACCAUCA CAGGGCAGCC 1450
 5 UAUGACAUUC CCCCCAGAGG CCCUGUGGGU GACCGUGGGG CUGUCUGUCU 1500
 GUCUCAUUG ACUCUGUGUG GCCCUGGCUU UCGUGUGCUG GAGAAAGAUC 1550
 10 AACAGAGCU GUGAGGAGGA GAAUGCAGGA GCUGAGGACC AGGAUGGGGA 1560
 GGGAGAAGG UCCAAGACAG CCCUGCAGCC UCUGAAACAC UCUGACAGCA 1600
 AAGAAGAUGA UGGACAAGAA AUAGCCUGAC CAUGAGGACC AGGGAGCUGC 1650
 15 UACCCCUCCC UACAGCUCCU ACCCUCUGGC UGC 1683

which is herein referred to as SEQ ID NO:5.

The ribonucleic acids of the present invention may be prepared using the polynucleotide synthetic methods discussed supra or they may be prepared enzymatically using any one of various RNA polymerases to transcribe a DNA template. This invention also provides nucleic acids, RNA or DNA, which are complementary to SEQ ID NO:1, SEQ ID NO:3, or SEQ ID NO:5

The present invention also provides probes and primers useful for molecular biology techniques. A compound which encodes for SEQ ID NO:1, SEQ ID NO:3, or SEQ ID NO:5; or a nucleic acid complimentary to SEQ ID NO:1, SEQ ID NO:3, or SEQ ID NO:5; or a fragment thereof and which is at least 18 base pairs in length, and which will selectively hybridize to genomic DNA or messenger RNA encoding a β -secretase protease, is provided. Preferably, the 18 or more base pair compound is DNA.

Primers and probes may be obtained by means well known in the art. For example, once a protein of interest is isolated, restriction enzymes and subsequent gel separation may be used to isolate the fragment of choice.

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The term "selectively hybridize" as used herein may refer to either of two situations. In the first such embodiment of this invention, the nucleic acid compounds described supra hybridize to DNA or RNA encoding a human β -secretase protease under more stringent hybridization conditions
5 than these same nucleic acid compounds would hybridize to nucleic acids encoding analogous β -secretases of another species. In the second such embodiment of this invention, these probes hybridize to the DNA or RNA encoding the β -secretase under more stringent hybridization conditions than other related compounds, including nucleic acid sequences encoding other
10 amyloid precursor protein proteases.

These probes and primers can be prepared enzymatically as described supra. In a most preferred embodiment, however, these probes and primers are synthesized using chemical means as described herein.

Those skilled in the art will recognize the techniques associated
15 with probes and primers are well known. For example, all or part of the probes or primers may be used to hybridize to the coding sequence. Then, through PCR amplification, the full length sequence may be generated. The full length sequence can be subsequently subcloned into any vector of choice. Alternatively, the primers or probes may be radioactively labeled at the 5' end
20 in order to screen cDNA libraries by conventional means. A primer or probe can be labeled with a radioactive element which provides for an adequate signal as a means for detection and has sufficient half-life to be useful for detection, such as ^{32}P , ^3H , ^{14}C or the like. Other materials which can be used to label the primer or probe include antigens that are recognized by a
25 specific labeled antibody, fluorescent compounds, enzymes, antibodies specific for a labeled antigen, and chemiluminescent compounds. An appropriate label can be selected having regard to the rate of hybridization and binding of the primer or probe to the nucleotide to be detected and the amount of nucleotide available for hybridization.

30 As discussed supra, recombinant DNA cloning vectors and

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expression vectors comprising the nucleic acids of the present invention can be prepared. Many such vectors are illustrated and described above. The preferred nucleic acid vectors are those which are DNA. The preferred recombinant DNA vectors comprise the isolated DNA sequences as given by
5 SEQ ID NO:1 or SEQ ID NO:3. The most preferred recombinant DNA vectors comprise the DNA sequence given by SEQ ID NO:3.

The skilled artisan understands that the type of cloning vector employed depends upon the availability of appropriate restriction sites, the type of host cell in which the vector is to be transfected or transformed, the
10 purpose of the transfection or transformation (e.g., transient expression in an oocyte system, stable transformation as an extrachromosomal element, or integration into the host chromosome), the presence or absence of readily assayable markers (e.g., antibiotic resistance markers, metabolic markers, or the like), and the number of copies of the gene to be present in the cell.

The type of vector employed to carry the nucleic acids of the present invention may be RNA viruses, DNA viruses, lytic bacteriophages, lysogenic bacteriophages, stable bacteriophages, plasmids, viroids, and the like. When preparing an expression vector, the skilled artisan understands that there are many variables to be considered. One such example is the use
20 of a constitutive promoter, i.e. a promoter which is functional at all times, instead of a regulatable promoter which may be activated or inactivated by the artisan using heat, addition or removal of a nutrient, addition of an antibiotic, and the like. The practitioner also understands that the amount of the nucleic acid or protein to be produced dictates, in part, the selection of the
25 expression system. For experiments examining the amount of the protein expressed on the cell membrane or for experiments examining the biological function of an expressed membrane protein, for example, it may be unwise to employ an expression system which produces too much of the protein. The addition or subtraction of certain sequences, such as a signal sequence
30 preceding the coding sequence, may be employed by the practitioner to

influence localization of the resulting polypeptide. Such sequences added to or removed from the nucleic acid compounds of the present invention are encompassed within this invention.

Plasmids can be readily modified to construct expression vectors
5 in a variety of organisms, including, but not limited to, E. coli, SF9 (as host for baculovirus), Spodoptera and Saccharomyces. See: Serook et al., Molecular Cloning: A Laboratory Manual (1989)

One of the most widely employed techniques for altering a
nucleic acid sequence is by way of oligonucleotide-directed site-specific
10 mutagenesis. B Comack, "Current Protocols in Molecular Biology", 8.01-8.5.9, (F. Ausubel, et al., eds. 1991). In this technique an oligonucleotide, whose sequence contains the mutation of interest, is synthesized as described supra. This oligonucleotide is then hybridized to a template containing the wild-type
sequence. In a most preferred embodiment of this technique, the template is
15 a single-stranded template. Particularly useful are plasmids which contain regions such as the f1 intergenic region. This region allows the generation of single-stranded templates when a helper phage is added to the culture harboring the "phagemid".

After the annealing of the oligonucleotide, to the template, a
20 DNA-dependent DNA polymerase is then used to synthesize the second strand from the oligonucleotide, complementary to the template DNA. The resulting product is a heteroduplex molecule containing a mismatch due to the mutation in the oligonucleotide. After DNA replication by the host cell a mixture of two types of plasmid are present, the wild-type and the newly
25 constructed mutant. This technique permits the introduction of convenient restriction sites such that the coding sequence may be placed immediately adjacent to whichever transcriptional or translational regulatory elements are employed by the practitioner.

The construction protocols utilized for E. coli can be followed to
30 construct analogous vectors for other organisms, merely by substituting, if

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necessary, the appropriate regulatory elements using techniques well know to skilled artisans.

Host cells which harbor the nucleic acids provided by the present invention can also be prepared. One suitable host cell is an
5 Xenopus sp. oocyte which has been injected with RNA or DNA compounds of the present invention. Preferred oocytes of the present invention are those which harbor a sense mRNA of the present invention. Other preferred host cells include HeLa and 293 cells which have been transfected and/or transformed with a vector which comprises a nucleic acid of the present
10 invention.

A method for constructing a recombinant host cell capable of expressing SEQ ID NO:2 or SEQ ID NO:4 is also possible with regard to the present invention, said method comprising transforming a host cell with a recombinant DNA vector that comprises an isolated DNA sequence which
15 encodes SEQ ID NO:2 or SEQ ID NO:4. The preferred host cell is 293 cells. A preferred vector for expression is one which comprises SEQ ID NO:1 or SEQ ID NO:3, most preferably SEQ ID NO:1. Transformed host cells may be cultured under conditions well known to skilled artisans such that SEQ ID NO: 2 or SEQ ID NO:4 are expressed, thereby producing the sequence of β -
20 secretase in the recombinant host cell.

A further embodiment of the invention consists of a method of isolating and purifying an amyloid precursor protein protease from a host cell expressing said protein. In this embodiment, a host cell, either prokaryotic or eukaryotic, expressing amyloid precursor protein protease, is cultured in an
25 appropriate medium until a substantial cell mass has been obtained. The second step of this embodiment is the isolation of amyloid precursor protein protease from the cultured cells. Two methods for purifying amyloid precursor protein protease from a non-transformed mammalian cell line are described in United States Patent Number 5,328,842, the entire contents of

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which are herein incorporated by reference. The following summarizes those methods.

Once grown and harvested, the cultured cells are lysed by nitrogen cavitation in the presence of protease inhibitors. A soluble fraction
5 is prepared from the lysate by ultracentrifugation. The resulting solution of cytosolic proteins contains β -secretase and is subjected to a series of purification procedures.

The soluble fraction of the cell lysate is run through a series of column chromatography procedures. Anion exchange chromatography is
10 followed by hydrophobic interaction, molecular sizing, and finally another hydrophobic interaction technique where the conditions are such that the β -secretase binds the resin weakly. Each column is run individually, and the eluate is collected in fractions while monitoring for absorbance at 280 nm. Fractions are assayed for β -secretase activity, and those fractions with the
15 desired activity are then run over the next column until a homogeneous solution of β -secretase is obtained.

Immunoaffinity purification using anti- β -secretase antibodies is an alternative to the series of chromatographic procedures already mentioned. Making antiserum or monoclonal antibodies directed against a
20 purified protein is well known in the art, and skilled artisans readily will be able to prepare anti- β -secretase antibodies. Preparing an immunoaffinity matrix using such antibodies and isolating β -secretase using the immunoaffinity matrix is also well within the skill of the art. See, AFFINITY CHROMATOGRAPHY PRINCIPLES & METHODS, Pharmacia Fine Chemicals, 1983.

25 The ability of an agent to inhibit the protein of the present invention is essential in the development of a multitude of indications. In developing agents which act as inhibitors of β -secretase, it would be desirable, therefore, to determine those agents which interact with the protein of the present invention. Generally, such an assay includes a method for
30 determining whether a substance is a functional ligand of β -secretase, said

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method comprising contacting a functional compound of the β -secretase with said substance, monitoring enzymatic activity by physically detectable means, and identifying those substances which effect a chosen response.

The instant invention provides such a screening system useful
5 for discovering agents which inhibit the β -secretase, said screening system comprising the steps of:

- a) isolating a β -secretase;
- b) exposing said β -secretase to a potential inhibitor of the β -secretase;
- 10 c) introducing a suitable substrate;
- d) quantifying the amount of cleavage of the substrate relative to a control in which no potential inhibitor has been introduced.

This allows one to rapidly screen for inhibitors of β -secretase.

15 Utilization of the screening system described above provides a sensitive and rapid means to determine compounds which inhibit β -secretase. This screening system may also be adapted to automated procedures such as a PANDEX® (Baxter-Dade Diagnostics) system allowing for efficient high-volume screening of potential therapeutic agents. In such a screening
20 protocol a protein protease is prepared as elsewhere described herein, preferably using recombinant DNA technology. A sample of a test compound is then introduced to the reaction vessel containing the protein protease followed by the addition of an appropriate substrate. In the alternative the substrate may be added simultaneously with the test compound.

25 The desirability of a bioactivity assay system which determines the response of β -secretase to a compound of interest is clear. The instant invention provides such a bioactivity assay, said assay comprising the steps of:

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- a) transfecting a mammalian host cell with an expression vector comprising DNA encoding β -secretase;
- 5 b) culturing said host cell under conditions such that the β -secretase protein is expressed;
- c) exposing said host cell so transfected to a test compound; and
- 10 d) measuring the change in a physiological condition known to be influenced by β -secretase relative to a control in which the transfected host cell is not exposed to a test compound.

The present invention comprises a method of using said

15 inhibitors of β -secretase in the treatment of patients with acquired disease states of the brain including Alzheimer's disease. As used herein, "patient" refers to a mammal such a rat, mouse, guinea pig, dog, or human. It is understood, however, that the preferred patient for use with the methods herein is a human patient. As used herein, the term "treatment" (or "treat"

20 or "treating") includes its generally accepted meaning which encompasses prohibiting, preventing, restraining, and slowing, stopping, or reversing the progression, severity, and resulting symptoms of acquired disease states of the brain. As such, the methods of the present invention encompass both therapeutic and prophylactic uses.

25 Compounds which are inhibitors of β -secretase are usually administered in the form of pharmaceutical compositions. These compounds can be administered by a variety of routes including oral, rectal, transdermal, subcutaneous, intravenous, intramuscular, and intranasal. These compounds are effective as both injectable and oral compositions. Such compositions are

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prepared in a manner well known in the pharmaceutical art and comprise at least one active compound.

The present invention also includes methods employing pharmaceutical compositions which contain, as the active ingredient, inhibitors of β -secretase associated with pharmaceutically acceptable carriers. In making the compositions of the present invention the active ingredient is usually mixed with an excipient, diluted by an excipient or enclosed within such a carrier which can be in the form of a capsule, sachet, paper or other container. When the excipient serves as a diluent, it can be a solid, semi-solid, or liquid material, which acts as a vehicle, carrier or medium for the active ingredient. Thus, the compositions can be in the form of tablets, pills, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosols (as a solid or in a liquid medium), ointments containing for example up to 10% by weight of the active compound, soft and hard gelatin capsules, suppositories, sterile injectable solutions, and sterile packaged powders.

In preparing a formulation, it may be necessary to mill the active compound to provide the appropriate particle size prior to combining with the other ingredients. If the active compound is substantially insoluble, it ordinarily is milled to a particle size of less than 200 mesh. If the active compound is substantially water soluble, the particle size is normally adjusted by milling to provide a substantially uniform distribution in the formulation, e.g. about 40 mesh.

Some examples of suitable excipients include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, water, syrup, and methyl cellulose. The formulations can additionally include: lubricating agents such as talc, magnesium stearate, and mineral oil; wetting agents; emulsifying and suspending agents; preserving agents such as methyl- and

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propylhydroxybenzoates; sweetening agents; and flavoring agents. The compositions of the invention can be formulated so as to provide quick, sustained or delayed release of the active ingredient after administration to the patient by employing procedures known in the art.

5 The compositions are preferably formulated in a unit dosage form, each dosage containing from about 5 to about 100 mg, more usually about 10 to about 30 mg, of the active ingredient. The term "unit dosage form" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined
10 quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical excipient.

 The active compound is effective over a wide dosage range. For examples, dosages per day normally fall within the range of about 0.5 to about 30 mg/kg of body weight. In the treatment of adult humans, the range
15 of about 1 to about 15 mg/kg/day, in single or divided dose, is especially preferred. However, it will be understood that the amount of the compound actually administered will be determined by a physician, in the light of the relevant circumstances, including the condition to be treated, the chosen route of administration, the actual compound administered, the age, weight,
20 and response of the individual patient, and the severity of the patient's symptoms, and therefore the above dosage ranges are not intended to limit the scope of the invention in any way. In some instances dosage levels below the lower limit of the aforesaid range may be more than adequate, while in other cases still larger doses may be employed without causing any harmful
25 side effect, provided that such larger doses are first divided into several smaller doses for administration throughout the day.

 For preparing solid compositions such as tablets the principal active ingredient is mixed with a pharmaceutical excipient to form a solid preformulation composition containing a homogeneous mixture of a
30 compound of the present invention. When referring to these preformulation

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compositions as homogeneous, it is meant that the active ingredient is dispersed evenly throughout the composition so that the composition may be readily subdivided into equally effective unit dosage forms such as tablets, pills and capsules. This solid preformulation is then subdivided into unit
5 dosage forms of the type described above containing from 0.1 to about 500 mg of the active ingredient of the present invention.

The tablets or pills of the present invention may be coated or otherwise compounded to provide a dosage form affording the advantage of prolonged action. For example, the tablet or pill can comprise an inner
10 dosage and an outer dosage component, the latter being in the form of an envelope over the former. The two components can be separated by enteric layer which serves to resist disintegration in the stomach and permit the inner component to pass intact into the duodenum or to be delayed in release. A variety of materials can be used for such enteric layers or coatings, such
15 materials including a number of polymeric acids and mixtures of polymeric acids with such materials as shellac, cetyl alcohol, and cellulose acetate.

The liquid forms in which the novel compositions of the present invention may be incorporated for administration orally or by injection include aqueous solutions, suitably flavored syrups, aqueous or oil
20 suspensions, and flavored emulsions with edible oils such as cottonseed oil, sesame oil, coconut oil, or peanut oil, as well as elixirs and similar pharmaceutical vehicles.

Compositions for inhalation or insufflation include solutions and suspensions in pharmaceutically acceptable, aqueous or organic solvents, or
25 mixtures thereof, and powders. The liquid or solid compositions may contain suitable pharmaceutically acceptable excipients as described supra. Preferably the compositions are administered by the oral or nasal respiratory route for local or systemic effect. Compositions in preferably pharmaceutically acceptable solvents may be nebulized by use of inert gases.
30 Nebulized solutions may be breathed directly from the nebulizing device or

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the nebulizing device may be attached to a face mask, tent, or intermittent positive pressure breathing machine. Solution, suspension, or powder compositions may be administered, preferably orally or nasally, from devices which deliver the formulation in an appropriate manner.

5 The following examples illustrate the pharmaceutical compositions of the present invention.

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Formulation Preparation 1

Hard gelatin capsules containing the following ingredients are prepared:

5

<u>Ingredient</u>	<u>Quantity</u> <u>(mg/capsule)</u>
Active Ingredient	30.0
10 Starch	305.0
Magnesium stearate	5.0

The above ingredients are mixed and filled into hard gelatin capsules in 340 mg quantities.

15

Formulation Preparation 2

A tablet formula is prepared using the ingredients below:

20

<u>Ingredient</u>	<u>Quantity</u> <u>(mg/tablet)</u>
Active Ingredient	25.0
25 Cellulose, microcrystalline	200.0
Colloidal silicon dioxide	10.0
Stearic acid 5.0	

30

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The components are blended and compressed to form tablets, each weighing 240 mg.

Formulation Preparation 3

5

A dry powder inhaler formulation is prepared containing the following components:

	<u>Ingredient</u>	<u>Weight %</u>
10	Active Ingredient	5
	Lactose	95

The active mixture is mixed with the lactose and the mixture is added to a dry powder inhaling appliance.

15

Formulation Preparation 4

Tablets, each containing 30 mg of active ingredient, are prepared as follows:

20

	<u>Ingredient</u>	<u>Quantity</u> <u>(mg/tablet)</u>
	Active Ingredient	30.0 mg
25	Starch	45.0 mg
	Microcrystalline cellulose	35.0 mg
	Polyvinylpyrrolidone	
30	(as 10% solution in water)	4.0 mg

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Sodium carboxymethyl starch	4.5 mg
Magnesium stearate	0.5 mg
Talc	<u>1.0 mg</u>
Total	120 mg

The active ingredient, starch and cellulose are passed through a No. 20 mesh U.S. sieve and mixed thoroughly. The solution of polyvinylpyrrolidone is mixed with the resultant powders, which are then passed through a 16 mesh U.S. sieve. The granules so produced are dried at 50-60°C and passed through a 16 mesh U.S. sieve. The sodium carboxymethyl starch, magnesium stearate, and talc, previously passed through a No. 30 mesh U.S. sieve, are then added to the granules which, after mixing, are compressed on a tablet machine to yield tablets each weighing 120 mg.

Formulation Preparation 5

Capsules, each containing 40 mg of medicament are made as follows:

<u>Ingredient</u>	<u>Quantity</u> <u>(mg/capsule)</u>
Active Ingredient	40.0 mg
Starch	109.0 mg

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Magnesium stearate	<u>1.0 mg</u>
--------------------	---------------

Total	150.0 mg
-------	----------

5 The active ingredient, cellulose, starch, and magnesium stearate are blended, passed through a No. 20 mesh U.S. sieve, and filled into hard gelatin capsules in 150 mg quantities.

Formulation Preparation 6

10

Suppositories, each containing 25 mg of active ingredient are made as follows:

	<u>Ingredient</u>	<u>Amount</u>
15	Active Ingredient	25 mg
	Saturated fatty acid glycerides to	2,000 mg

20 The active ingredient is passed through a No. 60 mesh U.S. sieve and suspended in the saturated fatty acid glycerides previously melted using the minimum heat necessary. The mixture is then poured into a suppository mold of nominal 2.0 g capacity and allowed to cool.

Formulation Preparation 7

25

Suspensions, each containing 50 mg of medicament per 5.0 ml dose are made as follows:

	<u>Ingredient</u>	<u>Amount</u>
30	Active Ingredient	50.0 mg

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	Xanthan gum	4.0 mg
	Sodium carboxymethyl cellulose (11%)	
5	Microcrystalline cellulose (89%)	50.0 mg
	Sucrose	1.75 g
	Sodium benzoate	10.0 mg
10	Flavor and Color	q.v.
	Purified water to	5.0 ml

15 The medicament, sucrose and xanthan gum are blended, passed through a No. 10 mesh U.S. sieve, and then mixed with a previously made solution of the microcrystalline cellulose and sodium carboxymethyl cellulose in water. The sodium benzoate, flavor, and color are diluted with some of the water and added with stirring. Sufficient water is then added to produce the

20 required volume.

Formulation Preparation 8

25 Capsules, each containing 15 mg of medicament, are made as follows:

	<u>Ingredient</u>	Quantity (mg/capsule)
30	Active Ingredient	15.0 mg

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PCT/US00/06707

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	Starch	407.0 mg
	Magnesium stearate	<u>3.0 mg</u>
5	Total	425.0 mg

The active ingredient, cellulose, starch, and magnesium stearate are blended, passed through a No. 20 mesh U.S. sieve, and filled into hard gelatin capsules in 425 mg quantities.

10

Formulation Preparation 9

An intravenous formulation may be prepared as follows:

15

	<u>Ingredient</u>	<u>Quantity</u>
	Active Ingredient	250.0 mg
	Isotonic saline	1000 ml

20

Formulation Preparation 10

A topical formulation may be prepared as follows:

25	<u>Ingredient</u>	<u>Quantity</u>
	Active Ingredient	1-10 g
	Emulsifying Wax	30 g
30	Liquid Paraffin	20 g

White Soft Paraffin to 100 g

The white soft paraffin is heated until molten. The liquid paraffin and
5 emulsifying wax are incorporated and stirred until dissolved. The active
ingredient is added and stirring is continued until dispersed. The mixture is
then cooled until solid.

Formulation Preparation 11

Sublingual or buccal tablets, each containing 10 mg of active ingredient, may be prepared as follows:

		Quantity
15	<u>Ingredient</u>	<u>Per Tablet</u>
	Active Ingredient(s)	10.0 mg
	Glycerol	210.5 mg
20	Water	143.0 mg
	Sodium Citrate	4.5 mg
	Polyvinyl Alcohol	26.5 mg
25	Polyvinylpyrrolidone	<u>15.5 mg</u>
	Total	410.0 mg

The glycerol, water, sodium citrate, polyvinyl alcohol, and
30 polyvinylpyrrolidone are admixed together by continuous stirring and

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maintaining the temperature at about 90°C. When the polymers have gone into solution, the solution is cooled to about 50-55°C and the medicament is slowly admixed. The homogenous mixture is poured into forms made of an inert material to produce a drug-containing diffusion matrix having a thickness of about 2-4 mm. This diffusion matrix is then cut to form individual tablets having the appropriate size.

Another preferred formulation employed in the methods of the present invention employs transdermal delivery devices ("patches"). Such transdermal patches may be used to provide continuous or discontinuous infusion of the compounds of the present invention in controlled amounts. The construction and use of transdermal patches for the delivery of pharmaceutical agents is well known in the art. See, e.g., U.S. Patent 5,023,252, issued June 11, 1991, herein incorporated by reference. Such patches may be constructed for continuous, pulsatile, or on demand delivery of pharmaceutical agents.

Frequently, it will be desirable or necessary to introduce the pharmaceutical composition to the brain, either directly or indirectly. Direct techniques usually involve placement of a drug delivery catheter into the host's ventricular system to bypass the blood-brain barrier. One such implantable delivery system, used for the transport of biological factors to specific anatomical regions of the body, is described in U.S. Patent 5,011,472, issued April 30, 1991, which is herein incorporated by reference.

Indirect techniques, which are generally preferred, usually involve formulating the compositions to provide for drug latentiation by the conversion of hydrophilic drugs into lipid-soluble drugs or prodrugs. Latentiation is generally achieved through blocking of the hydroxy, carbonyl, sulfate, and primary amine groups present on the drug to render the drug more lipid soluble and amenable to transportation across the blood-brain barrier. Alternatively, the delivery of hydrophilic drugs may be enhanced by

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intra-arterial infusion of hypertonic solutions which can transiently open the blood-brain barrier.

The proteins of this invention as well as fragments of these proteins may be used as antigens for the synthesis of antibodies. The term "antibody" as used herein describes antibodies, fragments of antibodies (such as, but not limited, to Fab, Fab', Fab₂', and Fv fragments), and chimeric, humanized, veneered, resurfaced, or CDR-grafted antibodies capable of binding antigens of a similar nature as the parent antibody molecule from which they are derived. The instant invention also encompasses single chain polypeptide binding molecules.

The term "antibody" as used herein is not limited by the manner in which the antibodies are produced, whether such production is in situ or not. The term "antibody" as used in this specification encompasses those antibodies produced by recombinant DNA technology means including, but not limited, to expression in bacteria, yeast, insect cell lines, or mammalian cell lines.

The production of antibodies, both monoclonal and polyclonal, in animals, especially mice, is well known in the art. See, e.g., C. Milstein, HANDBOOK OF EXPERIMENTAL IMMUNOLOGY, (Blackwell Scientific Pub., 1986); J. Goding, MONOCLONAL ANTIBODIES: PRINCIPLES AND PRACTICE, (Academic Press, 1983). For the production of monoclonal antibodies the basic process begins with injecting a mouse, or other suitable animal, with an immunogen. The mouse is subsequently sacrificed and cells taken from its spleen are fused with myeloma cells, resulting in a hybridoma that reproduces in vitro. The population of hybridomas is screened to isolate individual clones, each of which secretes a single antibody species, specific for the immunogen. The individual antibody species obtained in this way is each the product of a single B cell from the immune animal generated in response to a specific antigenic site, or epitope, recognized on the immunogenic substance.

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Chimeric antibodies are described in U.S. Patent No. 4,816,567, which issued March 28, 1989 to S. Cabilly, et al. This reference discloses methods and vectors for the preparation of chimeric antibodies. The entire contents of U.S. Patent No. 4,816,567 are herein incorporated by reference.

5 An alternative approach to production of genetically engineered antibodies is provided in U.S. Patent No. 4,816,397, which also issued March 28, 1989 to M. Boss, et al., the entire contents of which are herein incorporated by reference. The Boss patent teaches the simultaneous co-expression of the heavy and light chains of the antibody in the same host cell.

10 The approach of U.S. Patent 4,816,397 has been further refined as taught in European Patent Publication No. 0 239 400, which published September 30, 1987. The teachings of this European patent publication (Winter) are a preferred format for the genetic engineering of the reactive monoclonal antibodies of this invention. The Winter technology involves the
15 replacement of complementarity determining regions (CDRs) of a human antibody with the CDRs of a murine monoclonal antibody thereby converting the specificity of the human antibody to the specificity of the murine antibody which was the source of the CDR regions. This "CDR grafting" technology affords a molecule containing minimal murine sequence and thus is less
20 immunogenic.

Single chain antibody technology is yet another variety of genetically engineered antibody which is now well known in the art. See, e.g. R.E. Bird, et al., Science 242:423-426 (1988); PCT Publication No. WO 88/01649, which was published 10 March 1988; United States Patent
25 5,260,203, issued November 9, 1993, the entire contents of which are herein incorporated by reference. The single chain antibody technology involves joining the binding regions of heavy and light chains with a polypeptide sequence to generate a single polypeptide having the binding specificity of the antibody from which it was derived.

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The aforementioned genetic engineering approaches provide the skilled artisan with numerous means to generate molecules which retain the binding characteristics of the parental antibody while affording a less immunogenic format.

5 These antibodies are used in diagnostics, therapeutics or in diagnostic/therapeutic combinations. By "diagnostics" as used herein is meant testing that is related to either the in vitro or in vivo diagnosis of disease states or biological status in mammals, preferably in humans. By "therapeutics" and "therapeutic/diagnostic combinations" as used herein is
10 respectively meant the treatment or the diagnosis and treatment of disease states or biological status by the in vivo administration to mammals, preferably humans, of the antibodies of the present invention. The antibodies of the present invention are especially preferred in the diagnosis and/or
15 treatment of conditions associated with an excess or deficiency of β -secretase.

20

25

5 WHAT IS CLAIMED IS:

1. An isolated amino acid compound functional as an amyloid precursor protein protease which comprises the amino acid sequence given by SEQ ID NO:2 or SEQ ID NO:4, or a functional equivalent thereof, or a
10 fragment of at least 6 continuous amino acids thereof.
2. A nucleic acid compound encoding the amino acid compound of Claim 1.
- 15 3. A nucleic acid compound encoding the amino acid compound of Claim 1 which comprises the nucleic acid sequence given by SEQ ID NO:1 or SEQ ID NO:3.
- 20 4. A composition comprising an isolated nucleic acid compound containing a sequence encoding an amyloid precursor protein protease as claimed in Claim 2, wherein said sequence is selected from the group consisting of
 - (a) SEQ ID NO:1 or SEQ ID NO:3;
 - (b) nucleotides 25 through 1629 of SEQ ID NO:1
25 or SEQ ID NO: 3;
 - (c) SEQ ID NO:5;
 - (d) nucleotides 25 through 1629 of SEQ ID NO:5;
 - (e) a nucleic acid complimentary to (a), (b), (c), or
(d); and

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- (f) a fragment of (a), (b), (c), (d), or (e) that is at least 18 bases in length and which will selectively hybridize to human genomic DNA encoding an amyloid precursor protein protease.

5

5. The composition as claimed in Claim 4 wherein the isolated nucleic acid is deoxyribonucleic acid.

6. The composition as claimed in Claim 5 wherein the deoxyribonucleic acid is SEQ ID NO:1 or SEQ ID NO:3, or a sequence complimentary to SEQ ID NO:1 or SEQ ID NO:3.

7. The composition of Claim 5 wherein the deoxyribonucleic acid is nucleotides 25 through 1629 of SEQ ID NO:1 or SEQ ID NO:3, or a sequence complimentary to nucleotides 25 through 1629 of SEQ ID NO:1.

8. The composition as claimed in Claim 4 wherein the isolated nucleic acid is ribonucleic acid.

9. The composition as claimed in Claim 8 wherein the ribonucleic acid is SEQ ID NO:5 or a sequence complimentary to SEQ ID NO:5.

10. The composition of Claim 8 wherein the ribonucleic acid is nucleotides 25 through 1629 of SEQ ID NO:5 or a sequence complimentary to nucleotides 25 through 1629 of SEQ ID NO:5.

11. An expression vector capable of producing an amyloid precursor protein protease in a host cell which comprises a nucleic acid

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compound as claimed in Claim 4 in combination with regulatory elements necessary for expresison of the nucleic acid compound in a host cell.

12. An expression vector as claimed in Claim 11 for use in a host
5 cell wherein said host cell is a mammalian cell.

13. An expression vector as claimed in Claim 11 for use in a host
cell wherein said host cell is a prokaryotic cell.

14. A transfected host cell harboring an expression vector as
10 claimed in Claim 11.

15. A transfected host cell as claimed in Claim 14 wherein the
host cell is a mammalian cell.

16. A transfected host cell as claimed in Claim 14 wherein the
host cell is a prokaryotic cell.

17. A method to identify compounds which inhibit the synthesis
20 or release of beta-amyloid protien, which method comprises:

- (a) isolating a β -secretase;
- (b) exposing said β -secretase to a potential inhibitor of the β -secretase;
- (c) introducing a suitable substrate;
- 25 (d) quantifying the amount of cleavage of the substrate relative to a control in which no potential inhibitor has been introduced.

18. The method of Claim 17 wherein said β -secretase comprises the amino acid sequence given by SEQ ID NO:2 or SEQ ID NO:4.

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19. A method of evaluating the effectiveness of a test compound for the treatment or prevention of a condition associated with the synthesis or release of beta-amyloid protein, which method comprises:

- 5 (a) transfecting a mammalian host cell with an expression vector comprising DNA encoding β -secretase;
- (b) culturing said host cell under conditions such that the β -secretase protein is expressed;
- (c) exposing said host cell so transfected to a test compound; and
- 10 (d) measuring the change in a physiological condition known to be influenced by β -secretase relative to a control in which the transfected host cell is not exposed to a test compound.

20. The method of Claim 19 wherein said β -secretase comprises the amino acid sequence given by SEQ ID NO:2 or SEQ ID NO:4.

- 15 21. A method of treating or preventing Alzheimer's Disease or other neurodegenerative disorders in a patient which comprises administering to a patient in need thereof, an effective amount of an inhibitor of the amyloid precursor protein protease given by the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4.

- 20 22. A pharmaceutical composition comprising an inhibitor of the amyloid precursor protein protease given by SEQ ID NO:2 or SEQ ID NO:4, in combination with one or more pharmaceutically acceptable excipients, carriers, or diluents therefor.



DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION

☒ Declaration Submitted with Initial Filing
☐ Declaration Submitted after Initial Filing

Attorney Docket Number	X-11600
First Named Inventor	Gerald Wayne Becker, et al.
COMPLETE IF KNOWN	
Application Number	
Filing Date	
Group Art Unit	
Examiner Name	

As a below named Inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole Inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

AMYLOID PRECURSOR PROTEIN PROTEASE AND RELATED NUCLEIC ACID COMPOUNDS

the specification of which

☐ is attached hereto

OR

☒ was filed on
(MM/DD/YYYY)

05/09/2000

as United States Application Number or PCT International

Application
Number

PCT/US00/06707

and was amended on
(MM/DD/YYYY)

(if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37 Code of Federal Regulations, § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached	
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☐ Additional foreign application numbers are listed on a supplemental priority sheet attached hereto:

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below.

Application Number(s)	Filing Date (MM/DD/YYYY)	<input type="checkbox"/> Additional provisional application numbers are listed on a supplemental priority sheet attached hereto.
60/133,423	05/11/1999	

Please type a plus sign (+) inside this box



PTO/SB/01 (8-96) (MODIFIED)

Approved for use through 9/30/98, OMB 0651-0032

Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

DECLARATION

I hereby claim the benefit under Title 35, United States Code §120 of any United States application(s), or § 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of Title 35, United States Code § 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. Parent Application Number	PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number (if applicable)

☐ Additional U.S. or PCT international application numbers are listed on a supplemental priority sheet attached hereto.

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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PTO/SB/01 (8-96) (MODIFIED)

Approved for use through 9/30/98. OMB 0651-0032
Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

DECLARATION

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Approved for use through 9/30/98. OMB 0651-0032
Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

DECLARATION

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atg ggt gtg cat gtg ggt gca gcc ctg gga gca ctg tgg ttc tgc ctc	99
Met Gly Val His Val Gly Ala Ala Leu Gly Ala Leu Trp Phe Cys Leu	
10 15 20 25	
aca gga gcc ctg gag gtc cag gtc cct gaa gac cca gtg gtg gca ctg	147
Thr Gly Ala Leu Glu Val Gln Val Pro Glu Asp Pro Val Val Ala Leu	
30 35 40	
gtg ggc acc gat gcc acc ctg tgc tgc tcc ttc tcc cct gag cct ggc	195
Val Gly Thr Asp Ala Thr Leu Cys Cys Ser Phe Ser Pro Glu Pro Gly	
45 50 55	
ttc agc ctg gca cag ctc aac ctc atc tgg cag ctg aca gat acc aaa	243
Phe Ser Leu Ala Gln Leu Asn Leu Ile Trp Gln Leu Thr Asp Thr Lys	
60 65 70	
cag ctg gtg cac agc ttt gct gag ggc cag gac cag ggc agc gcc tat	291
Gln Leu Val His Ser Phe Ala Glu Gly Gln Asp Gln Gly Ser Ala Tyr	

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75				80				85								
gcc Ala 90	aac Asn	cgc Arg	acg Thr	gcc Ala	ctc Leu 95	ttc Phe	cgg Pro	gac Asp	ctg Leu	ctg Leu 100	gca Ala	cag Gln	ggc Gly	aac Asn	gca Ala 105	339
tcc Ser	ctg Leu	agg Arg	ctg Leu	cag Gln 110	cgc Arg	gtg Val	cgt Arg	gtg Val	cgc Ala 115	gac Asp	gag Glu	ggc Gly	agc Ser	ttc Phe 120	acc Thr	387
tgc Cys	ttc Phe	gtg Val	agc Ser 125	atc Ile	cgg Arg	gat Asp	ttc Phe	ggc Gly 130	agc Ser	gct Ala	gcc Ala	gtc Val	agc Ser 135	ctg Leu	cag Gln	435
gtg Val	gcc Ala	gct Ala 140	ccc Pro	tac Tyr	tcg Ser	aag Lys	ccc Pro 145	agc Ser	atg Met	acc Thr	ctg Leu	gag Glu 150	ccc Pro	aac Asn	aag Lys	483
gac Asp	ctg Leu 155	cgg Arg	cca Pro	ggg Gly	gac Asp	acg Thr 160	gtg Val	acc Thr	atc Ile	acg Thr	tgc Cys 165	tcc Ser	agc Ser	tac Tyr	cag Gln	531
ggc Gly 170	tac Tyr	cct Pro	gag Glu	gct Ala	gag Glu 175	gtg Val	ttc Phe	tgg Trp	cag Gln	gat Asp 180	ggg Gly	cag Gln	ggc Gly	gtg Val	ccc Pro 185	579
ctg Leu	act Thr	ggc Gly	aac Asn	gtg Val 190	acc Thr	acg Thr	tcg Ser	cag Gln	atg Met 195	gcc Ala	aac Asn	gag Glu	cag Gln	ggc Gly 200	ttg Leu	627
ttt Phe	gat Asp	gtg Val	cac His 205	agc Ser	atc Ile	ctg Leu	cgg Arg	gtg Val 210	gtg Val	ctg Leu	ggc Gly	gca Ala	aat Asn 215	ggc Gly	acc Thr	675
tac Tyr	agc Ser	tgc Cys 220	ctg Leu	gtg Val	cgc Arg	aac Asn	ccc Pro 225	gtg Val	ctg Leu	cag Gln	cag Gln	gat Asp 230	gcg Ala	cac His	agc Ser	723
tct Ser	gtc Val 235	acc Thr	atc Ile	aca Thr	ccc Pro	cag Gln 240	aga Arg	agc Ser	ccc Pro	aca Thr	gga Gly 245	gcc Ala	gtg Val	gag Glu	gtc Val	771
cag Gln 250	gtc Val	cct Pro	gag Glu	gac Asp	cgg Pro 255	gtg Val	gtg Val	gcc Ala	cta Leu	gtg Val 260	ggc Gly	acc Thr	gat Asp	gcc Ala	acc Thr 265	819
ctg Leu	cgc Arg	tgc Cys	tcc Ser	ttc Phe 270	tcc Ser	ccc Pro	gag Glu	cct Pro	ggc Gly 275	ttc Phe	agc Ser	ctg Leu	gca Ala	cag Gln 280	ctc Leu	867
aac Asn	ctc Leu	atc Ile	tgg Trp 285	cag Gln	ctg Leu	aca Thr	gac Asp	acc Thr 290	aaa Lys	cag Gln	ctg Leu	gtg Val	cac His 295	agt Ser	ttc Phe	915
acc Thr	gaa Glu	ggc Gly 300	cgg Arg	gac Asp	cag Gln	ggc Gly	agc Ser 305	gcc Ala	tat Tyr	gcc Ala	aac Asn	cgc Arg 310	acg Thr	gcc Ala	ctc Leu	963

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ttc ccg gac ctg ctg gca caa ggc aat gca tcc ctg agg ctg cag cgc	1011
Phe Pro Asp Leu Leu Ala Gln Gly Asn Ala Ser Leu Arg Leu Gln Arg	
315 320 325	
gtg cgt gtg gcg gac gag ggc agc ttc acc tgc ttc gtg agc atc cgg	1059
Val Arg Val Ala Asp Glu Gly Ser Phe Thr Cys Phe Val Ser Ile Arg	
330 335 340 345	
gat ttc ggc agc gct gcc gtc agc ctg cag gtg gcc gct ccc tac tcg	1107
Asp Phe Gly Ser Ala Ala Val Ser Leu Gln Val Ala Ala Pro Tyr Ser	
350 355 360	
aag ccc agc atg acc ctg gag ccc aac aag gac ctg cgg cca ggg gac	1155
Lys Pro Ser Met Thr Leu Glu Pro Asn Lys Asp Leu Arg Pro Gly Asp	
365 370 375	
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Thr Val Thr Ile Thr Cys Ser Ser Tyr Arg Gly Tyr Pro Glu Ala Glu	
380 385 390	
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Val Phe Trp Gln Asp Gly Gln Gly Val Pro Leu Thr Gly Asn Val Thr	
395 400 405	
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Thr Ser Gln Met Ala Asn Glu Gln Gly Leu Phe Asp Val His Ser Val	
410 415 420 425	
ctg cgg gtg gtg ctg ggt gcg aat ggc acc tac agc tgc ctg gtg cgc	1347
Leu Arg Val Val Leu Gly Ala Asn Gly Thr Tyr Ser Cys Leu Val Arg	
430 435 440	
aac ccc gtg ctg cag cag gat gcg cac ggc tct gtc acc atc aca ggg	1395
Asn Pro Val Leu Gln Gln Asp Ala His Gly Ser Val Thr Ile Thr Gly	
445 450 455	
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Gln Pro Met Thr Phe Pro Pro Ala Leu Trp Val Thr Val Gly Leu	
460 465 470	
tct gtc tgt ctc att gca ctg ctg gtg gcc ctg gct ttc gtg tgc tgg	1491
Ser Val Cys Leu Ile Ala Leu Leu Val Ala Leu Ala Phe Val Cys Trp	
475 480 485	
aga aag atc aaa cag agc tgt gag gag gag aat gca gga gct gag gac	1539
Arg Lys Ile Lys Gln Ser Cys Glu Glu Glu Asn Ala Gly Ala Glu Asp	
490 495 500 505	
cag gat ggg gag gga gaa ggc tcc aag aca gcc ctg cag cct ctg aaa	1587
Gln Asp Gly Glu Gly Glu Gly Ser Lys Thr Ala Leu Gln Pro Leu Lys	
510 515 520	
cac tct gac agc aaa gaa gat gat gga caa gaa ata gcc tga	1629
His Ser Asp Ser Lys Glu Asp Asp Gly Gln Glu Ile Ala	
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ccatgaggac cagggagctg ctacccctcc ctacagctcc taccctctgg ctgc 1683

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35 40 45

Cys Cys Ser Phe Ser Pro Glu Pro Gly Phe Ser Leu Ala Gln Leu Asn
50 55 60

Leu Ile Trp Gln Leu Thr Asp Thr Lys Gln Leu Val His Ser Phe Ala
65 70 75 80

Glu Gly Gln Asp Gln Gly Ser Ala Tyr Ala Asn Arg Thr Ala Leu Phe
85 90 95

Pro Asp Leu Leu Ala Gln Gly Asn Ala Ser Leu Arg Leu Gln Arg Val
100 105 110

Arg Val Ala Asp Glu Gly Ser Phe Thr Cys Phe Val Ser Ile Arg Asp
115 120 125

Phe Gly Ser Ala Ala Val Ser Leu Gln Val Ala Ala Pro Tyr Ser Lys
130 135 140

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Pro Asn Lys Asp Leu Arg Pro Gly Asp Thr Val Thr Ile Thr Cys Ser
370 375 380

Ser Tyr Arg Gly Tyr Pro Glu Ala Glu Val Phe Trp Gln Asp Gly Gln
385 390 395 400

Gly Val Pro Leu Thr Gly Asn Val Thr Thr Ser Gln Met Ala Asn Glu
405 410 415

Gln Gly Leu Phe Asp Val His Ser Val Leu Arg Val Val Leu Gly Ala
420 425 430

Asn Gly Thr Tyr Ser Cys Leu Val Arg Asn Pro Val Leu Gln Gln Asp
435 440 445

Ala His Gly Ser Val Thr Ile Thr Gly Gln Pro Met Thr Phe Pro Pro
450 455 460

Glu Ala Leu Trp Val Thr Val Gly Leu Ser Val Cys Leu Ile Ala Leu
465 470 475 480

Leu Val Ala Leu Ala Phe Val Cys Trp Arg Lys Ile Lys Gln Ser Cys
485 490 495

Glu Glu Glu Asn Ala Gly Ala Glu Asp Gln Asp Gly Glu Gly Glu Gly
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Ser Lys Thr Ala Leu Gln Pro Leu Lys His Ser Asp Ser Lys Glu Asp
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• " •

10010503 . 05000

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